A STUDY ON CHRONIC AUTOIMMUNE URTICARIA AND EFFICACY OF AUTOLOGOUS SERUM THERAPY

Dissertation Submitted in

fulfilment of the university regulations for

MD DEGREE IN

DERMATOLOGY, VENEREOLOGY AND LEPROLOGY

(BRANCH XX)

THE TAMILNADU DR. M. G. R. MEDICAL UNIVERSITY

CHENNAI

APRIL – 2012

CERTIFICATE

This is to certify that the dissertation entitled **"A STUDY ON CHRONIC AUTOIMMUNE URTICARIA AND EFFICACY OF AUTOLOGOUS SERUM THERAPY''** is a bonafide work done by **Dr. S. D. Sindhuja.**, at Madras Medical College, Chennai in partial fulfilment of the university rules and regulations for award of M.D., Degree in Dermatology, Venereology and Leprology (Branch-XX) under my guidance and supervision during the academic year 2009 -2012.

Prof. Dr. S.Jayakumar, M.D., D.D.,
Professor and Head,
Department of Dermatology and Leprosy,
Madras Medical College,
Rajiv Gandhi Govt. General Hospital,
Chennai-600 003.

Prof. Dr. V.Kanagasabai, M.D.,
Dean,
Madras Medical College &
Rajiv Gandhi Govt. General Hospital,
Chennai-600003

DECLARATION

I, **DR. S. D. SINDHUJA,** solemnly declare that dissertation titled, "**A STUDY ON CHRONIC AUTOIMMUNE URTICARIA AND EFFICACY OF AUTOLOGOUS SERUM THERAPY"** is a bonafide work done by me at Department of Dermatology and Leprosy, Madras Medical College, Chennai-3 during the period of October 2009 to September 2011 under the supervision of **Prof. DR. S. JAYAKUMAR, M.D., D.D,** Professor and HOD, The Department of Dermatology and Leprosy, Madras Medical College, Chennai. The dissertation is submitted to Tamilnadu Dr. M.G.R. Medical University, towards partial fulfilment of requirement for the award of **M.D. Degree (Branch-XX) in DERMATOLOGY, VENEREOLOGY AND LEPROLOGY**.

(Signature of the candidate)

Place: Chennai

Date:

SPECIAL ACKNOWLEDGEMENT

My sincere thanks to **Prof. V. KANAGASABAI, M.D.,** Dean, Madras Medical College for allowing me to do this dissertation and utilize the institutional facilities.

ACKNOWLEDGEMENT

I am gratefully indebted to **Prof. Dr. S. JAYAKUMAR, M.D., D.D.,** Professor and Head, Department of Dermatology and Leprosy for his invaluable guidance, motivation and help throughout the study. I would like to express my sincere and heartful gratitude to **Prof. Dr. V. SUDHA, M.D., D.V. D. D,** Director, Institute of Venereology.

I wish to thank Prof. **Dr. D. PRABHAVATHY, M.D., D.D.,** Former Professor and Head, Department of Dermatology and Leprosy for her constant support and motivation. I wish to thank Prof. **Dr. GAJENDRAN, M.D., D.V.,** Former Director, Institute of Venereology for his constant support and motivation. I express my gratefulness to Prof. **Dr. V.SOMASUNDARAM, M.D., D.D.,** Former Professor and Head of Department of Occupational Dermatology and Contact Dermatitis for his constant motivation and guidance.

I express my sincere gratitude to **Prof. Dr. R. ARUNADEVI, M.D.,D.D.,** Professor of Leprosy for her support. I am very grateful to **Prof. Dr. C. JANAKI M.D.,D.D.,** Department of Dermatology (Mycology) for her support. I sincerely thank **Prof. Dr. V. SAMPATH, M.D.,** Department of Dermatology for his priceless support. I thank **Prof. Dr. S. NIRMALA, M.D., D.D.,** for her immense help. I Thank **Prof. Dr. R. PRIYAVATHANI, M.D.,** for her support.

I specially thank **DR. SAMUEL JAYARAJ DANIEL, M.D (D.V.L).,** Assistant Professor, Department of Dermatology for his immense guidance and support.

I incline to thank **DR. G. K. THARINI, M.D., DR. S. MADHU, M.D., D.C.H.,** and **DR. C. VIJAYABASKAR, M.D., D.C.H.,** Assistant Professors, Department of Dermatology for their kind support and encouragement.

I thank **DR. J. MANJULA, M.D., D.N.B., DR. AFTHAB JAMEELA WAHAB, M.D.,D.D.,** and **DR. S. SARAVANAN, M.D.,** Assistant Professors, Department of Occupational Dermatology and Contact Dermatitis for their support and help.

My sincere thanks to **DR. P. ELANGOVAN, M.D., D.V.,** for his valuable guidance. I thank **DR. K. VENKATESWARAN, M.D., D.V., DR. S. THILAGAVATHY, M.D., D.V., DR. P. MOHAN, M.D., D.V., DR. S. ARUNKUMAR, M.D., D.V., DR. S. KALAIVANI, M.D., D.V., DR. S. PRABAHAR, M.D(D.V.L)., DR. V.N.S. AHAMED SHARIFF M.D (D.V.L).,** Assistant Professors, Institute of Venereology for their help and suggestions.

I duly acknowledge the paramedical staff and my colleagues for their help and favour.

Last but not the least I am profoundly grateful to all patients for their cooperation and participation in the study.

CONTENTS

Introduction

INTRODUCTION

Urticaria is a distressing dermatosis of skin characterized by transient erythematous edematous weals which are very itchy.

Urticaria, commonly called 'hives,' has a long and rich history in documented medicine dating back to the 10th century B.C[1]. Chronic idiopathic urticaria (CIU) is defined as wide spread, short lived (< 24 h) weals occurring daily or almost daily for at least 6 weeks, with no obvious cause. Activation of cutaneous mast cells liberates various mediators predominantly histamine which induces increased permeability of capillaries and venules which in turn produces uriticaria.

It is now recognized that approximately 30-40% of patients with CIU have histamine-releasing autoantibodies directed against either the high-affinity IgE receptor, or less frequently, IgE. This subset of patients are categorized as having chronic autoimmune urticaria. The presence of autoantibodies may be important clinically in severely affected, treatment-resistant patients, where immunomodulatory treatments may be helpful[2].

The ASST (Autologous Serum Skin Test) is a simple invivo screening test for detecting patients with autoimmunity[3]. It has a sensitivity of 70% and a specificity of 80%. A positive test is suggestive but not diagnostic of an autoimmune basis. Confirmation is needed by invitro

testing of the patient's serum for the anti-Fc€RIα or the anti-IgE auto antibodies. Basophil histamine release assay is the gold standard for detecting functional autoantibodies. However, it is available only at a few research centers and cannot be performed as a routine[4]. CIU is extremely disabling in its severe form and can be difficult to treat. Autoimmune urticaria patients with severe disease are at times refractory to treatment with conventional antihistamines and need immunomodulators. Autologous Serum Therapy (AST), a modified form of autologous whole blood therapy, has been found to be fairly effective in chronic urticaria[5].

Review of literature

REVIEW OF LITERATURE

History

Earliest description of the disease is found in “The Yellow Emperor’s Inner Classic”, Huang Di Nei Jing written between 1000-200 BC[6]. Hippocrates described the elevated itchy skin lesions caused by stings of nettles and mosquitoes and named it as “Knidosis” after the Greek word for nettle (knido). The word urticaria was first introduced in 1769 by William Cullen in his book “Synopsia Nosalogiae Methodica"[7]. Terms like 'Uredo,' 'essera' (Arabic for elevation) and ‘urticatio’ (derived from the Latin *urere*; to burn) have all been used previously[8] . In 1986 Grattan et al noted that injection of autologous serum intradermally elicited an immediate type of weal and flare reaction indicative of circulating mast cell activating factors[9.] In 1993, hide et al reported the presence of functional auto antibodies against high affinity receptor of IgE, Fc€RIα causing histamine release in a subset of chronic urticaria patients[10].

Epidemiology

Urticaria is a worldwide disease and may present at any age. There is no racial variance in the incidence. In one study point prevalence of 0.1% is noted. The lifetime occurrence of urticaria in the general population ranges from 1% to 5%[11]. Among chronic idiopathic urticaria patients,

around 40-60% are found to have chronic autoimmune urticartia[12].

Classification

Urticaria is broadly classified into:

Ordinary Urticaria

- Acute
- Episodic (acute and chronic intermittent)
- Chronic

Physical

- Dermographism
- Delayed pressure urticaria
- Cholinergic urticaria
- Cold urticaria
- Solar urticaria
- Aquagenic urticaria
- Heat urticaria
- Vibratory angio odema
- Urticarial vasculitis
- Contact urticaria
- Angioedema without weals

Other syndromes resembling urticaria or angioedema or with urticaria as a component[12].

Acute and chronic urticaria are classified based on the time duration. When symptoms are present for less than 6 weeks duration it is called acute urticaria. If it appears almost daily, continuously for 6 or more weeks it is termed as chronic urticaria[12].

Chronic autoimmune Urticaria

Sera of approximately 60% of patients with chronic ordinary urticaria have shown to cause a weal when injected intradermally into patients own skin. This indicates presence of circulating autoantibodies in the blood. This group of patients are categorized as having chronic autoimmune urticaria [3,5,12].

Genetics

Hereditary angiodema, Muckle wells syndrome and few rare types of physical urticaria are inherited as autosomal dominant traits. HLA DR4 and HLA DR8 are associated with chronic autoimmune urticaria[13].

Causes of urticaria

1. Autoimmunity

Before the detection of functional autoantibodies which release histamine from mast cells, majority of chronic urticaria patients were categorized as having chronic idiopathic urticaria. These antibodies denote

the presence of autoimmune etiology in an important subset of patients, now classified as chronic autoimmune urticaria[12].

Evidence for the presence of autoimmune etiology has been concluded based on various investigations:

1. Occurrence of weal and flare on intradermal injection with patient's own serum[9].

2. Serum and purified IgG derived from this subset of patients was able to release histamine from mast cells and basophils in invitro tests.

3. The plasma level of these antibodies detected using ELISA, Immunoblot assays varied in proportion with the disease activity[14].

4. Clinical remission has occurred in patients following plasmapheresis to remove these autoantibodies[15].

2. ALLERGIC ETIOLOGY

Allergic reactions are only rarely responsible for chronic urticaria[16,17]. It is an IgE mediated reaction. It occurs in an already sensitized individual where preformed specific IgE are bound to the mast cells. On repeated exposure, the allergen interacts with the IgE on mast cells to release mediators. It occurs within minutes and not longer than 60 minutes[12]. The offending substance is easily recognized by the patient and

avoided. Various drugs, food, injection and implants can be a potential allergen.

3. NON ALLERGIC:

Direct mast cell liberators:

Substances like morphine, codeine, atracurium and antibiotics, such as polymyxin and vancomycin can liberate histamine from mast cells directly. It is not immunologically mediated and previous sensitization is not required. It may occur after first exposure[12].

Pseudo allergic reactions:

Drugs like aspirin and other non steroidal anti inflammatory drugs cause urticaria by inhibiting cyclooxygenase pathway and increased diversion to lipooxygenase pathway resulting in increased production of proinflammatory mediators like LTC4, LTD4, LTE4. It also reduces PGE2 which is an inhibitor of immunological mast cell degranulation. Pseudoallergic reactions is not substance specific and are related to the dose of the offending substance[18].

4. Drugs

Many drugs cause urticaria through allergic or non allergic mechanisms[19, 20]. Penicillins, cephalosporins, sulfonamides and

tetracyclines are some examples of drug induced urticaria. Drug which is taken for a long time is unlikely to cause urticaria. On the contrary even a small quantity of penicillin present in the dairy products may produce severe urticaria in a sensitive person[21].

5. Infections

Infections may produce either acute or chronic urticaria. Hepatitis B viral infection, streptococcal throat infection, campylobacter jejuni and non specific viral infections are few examples which can produce acute urticaria. Bacterial infections of the dental, throat, respiratory, urinary tracts, gall bladder and Helicobacter pylori rarely may be responsible for chronic urticaria[22, 23].

6. Infestations

Gastro-intestinal parasites like Ankylostoma, Strongyloides, Echinococcus and Toxacara canis can cause urticaria. However this has become a rare cause as shown in recent Indian studies[24].Ancylostoma and Strongyloides worms migration can cause linear weals[12]. Toxacara canis antibodies have been found to be associated with chronic urticaria, but a causal relationship is not proven[25].

7. Inhalants

Various substances like grass, pollens, mould, spores, animal danders, and house dust may cause acute or chronic urticaria with or without respiratory symptoms[26].

8. Ingestion

Various food and food additives can cause both acute and chronic urticaria. They cause urticaria by vasoactive amines present in them (cheese, tomatoes, fish, meat, pineapples and avocados) or by the presence of histamine releasing substances (strawberries). Fish, milk, peanut, beans, potato, rice, carrot and drumstick are responsible for acute urticaria mediated through IgE dependant mechanism[27]. The reaction can occur in a few minutes to many hours. Food additives like tartrazine, dyes like sunset yellow, food preservatives like benzoate preservatives, antioxidants like butylated hydroxytoluene and dyes used in medicinal products can all cause urticaria. Among the alcoholic beverages, red wine which contains vasoactive amines like histamine and white wine treated with sulphite may cause urticaria in some[28].

9. Insect bites

Bite of insects like mosquitoes and bed bugs can cause chronic and recurrent urticaria. Wasp or bee stings may produce severe acute urticaria

or anaphylaxis through allergic mechanism which may be life threatening[29].

10. Implant

Dental prosthesis, dental amalgams and metal pins used in orthopaedics practice may rarely cause urticaria[30].

11. Others

Urticaria may occur in pregnancy and premenstrual exacerbation can occur. Predominant premenstrual occurrence is linked to the sensitivity to progesterone or estrogen on an autoimmune basis[31]. Psychological stress can flare up urticaria[32]. Depression and anxiety were frequently found in chronic urticaria patients. Depression has been found to reduce the threshold for pruritus[33].

It is still not possible to identify the cause of urticaria in around 50% of patients. These patients are categorized as having chronic idiopathic urticaria[12].

Other aggravating factors include upper respiratory tract infections, pressure, overheating, alcohol and viral infections.

PATHOGENESIS

Mast cell is the principal effector cell of urticaria[12]. Degranulation of mast cells can occur following immunological or non immunological stimuli.

Immunological stimuli are seen in autoimmune and allergen mediated urticaria. Fc€RIα are high affinity IgE receptors expressed on mast cells. The constant region domain of IgE, C_H3, is the major site of interaction with the IgE receptor on mast cells. When allergen binds to this IgE receptor complex on mast cell, or when cross linking of adjacent Fc€RIα occurs, there is activation and phosphorylation of protein tyrosine kinase and phospholipase C. This induces the production of second messengers like IP3 and DAG. IP3 increases intracellular calcium which promotes the assembly of microtubules and the contraction of microfilaments, both of which cause movement of granules to the plasma membrane. Fusion of the granules with plasma membrane occurs with the help of SNARE protein resulting in degranulation by exocytosis[34].

Non-immunologic stimuli such as opioids, C5a anaphylotoxin, stem cell factor as well as neuropeptides such as substance P can cause degranulation via direct stimulation. These stimuli initiate calcium and energy dependent steps that cause storage granules to fuse with the cell

membrane and externalize their contents, which include preformed and newly synthesized mediators of inflammation. Fc€RIα stimulation also leads to upregulation of the synthesis and secretion of proinflammatory mediators[35].

Non mast cell dependent urticaria occur in C1 esterase inhibitor deficiency (due to increased bradykinin) and in urticaria due to use of angiotensin converting enzyme inhibitors (inhibition of breakdown of bradykinin by angiotensin converting enzyme) [12].

Mast cell degranulation initiates the inflammatory process. The recruitment of inflammatory cells requires presence of cytokines and chemotactic factors which cause activation, expression of adhesion molecules and recruitment of cells. Sources of these chemokines include the mast cell and the activated endothelial cell .Endothelial cell activation is suggested by the presence of intercellular adhesion molecule 1 and E-selectin in biopsy specimens of urticarial lesions[36].

On stimulation, mast cells release both preformed mediators and newly generated mediators.

Preformed mediators: heparin, histamine, proteases like tryptase and chymase[37, 12].

Newly generated: leukotrienes C4, D4, E4, platelet activating factor and prostaglandin D2.

Cytokines: TNFα, IL3, IL4, IL5, IL6, IL8, IL13 and GM-CSF

Basophils also express Fc€RIα and release mediators on activation like histamine, IL4, IL13, LTC4[12].

Histamine

Histamine is the most important preformed mediator in the pathophysiology of urticaria. It is released from mast cells following degranulation.Increased concentration of histamine has been recovered in tissue fluid from lesions of urticaria. Histamine release from mast cells is more in chronic urticaria patients compared to normal subjects. There is no evidence for decreased skin histamine metabolism in chronic urticaria but cutaneous vasculature of these patients is more responsive to histamine than skin of normal controls[38].

Histamine after its release acts on its receptors. Activation of H1 receptor in the skin induces itching, flare, erythema and wealing. H2 receptors activation causes erythema and wealing but not itch or flare. So far, H3 receptors which are inhibitory autoreceptors present in nervous system have not been identified in human skin[12].

Other mediators

Tryptase and chymase are preformed mediators released along with histamine. Tryptase mediate itch by its effect on activation of

proteinase activated receptor 2 present on sensory nerve fibre and endothelial cells[39]. Chymase can induce mast cell degranulation. Tryptase and chymase cleave C3 to C3a and C3b. C3a can activate mast cells and C3b activates alternate complement pathway[12].

TNFα up regulates vascular endothelial adhesion molecules. IL8 stimulates neutrophil activation and accumulation. Some mast cells are positioned close to nerve endings where histamine stimulates sensory afferent nerves to release substance P. Neuropeptides appear to be responsible for the neurogenic axon flare after intradermal histamine injection[12].

Cellular involvement

The dermal cellular infiltrate consists of lymphocytes, neutrophils and eosinophils. They release a variety of cytokines with pro inflammatory properties. The observation that the cellular infiltrate in weals of patients with chronic autoimmune and idiopathic urticaria are similar suggests that the inflammatory response is determined by the event of mast cells degranulation rather than its stimulus[40].

Blood basophil levels are reduced in chronic urticaria. They are actively recruited from peripheral blood into lesional skin by expression of endothelial adhesion molecules, including VCAM-1, induced by

degranulation of lesional skin mast cells. Basophil releasability of mediators is found to be reduced in chronic autoimmune urticaria patients with antiIgE and anti Fc€RIα antibodies implying that circulating basophils in these patients may be in a state of desensitization from further stimulation through the Fc€RIα [41, 43]. But this did not apply to skin mast cells. Basophils also contribute to prolonged duration of weals[42].

Autoimmunity

Autoantibodies play a major role in the pathogenesis of patients with chronic autoimmune urticaria. These are IgG antibodies directed against

1. Fc€RIα receptor on mast cell in 30-40%
2. IgE bound to Fc€RIα receptor in 5-10%[43, 2, 44].

Anti Fc€RIα

These autoantibodies react with the α-subunit of the human high-affinity immunoglobulin E receptor (Fc€RIα). Detection of these antibodies even in normal healthy individuals indicates that they are part of natural antibody repertoire unit of immune system[45]. What makes these antibodies functional in releasing antibodies by certain set of patients has been a field of interest. Suggested theories include,

Presence of anti idiotype antibodies to the anti Fc€RIα autoantibodies in healthy individuals and loss of this balance in autoimmune urticaria patients, rendering them functional to release mediators. Treatment with IVIG which contains natural antibodies is believed to restore the unbalanced anti idiotype network and improvement of symptoms[46].

Mechanism of conditional autoimmunity was proposed where various factors influence the function of these autoantibodies[47]. The Fc€RIα receptors are occupied by IgE which blocks the binding of anti-Fc€RIα autoantibodies to the receptors. When receptor is not occupied by IgE, the autoantibodies bind and release mediators. Factors influencing this receptor occupancy include,

1. Imbalance between the level of anti- Fc€RIα antibodies and IgE.

2. Amounts of free IgE bound to secreted Fc€RIα chain.

3. Receptor density may be up regulated or down regulated by levels of serum IgE.

4. Actual numbers of cells, and therefore receptors may be increased for example, during an ongoing allergic inflammation[47].

Anti IgE

These antibodies are detected in 5-10% of autoimmune urticaria patients. All these antibodies cause mediator release from mast cells and basophils by cross linking of either the receptor or the IgE attached to the receptor. Autoantibodies to mast cells may also initiate complement activation with generation of C5a anaphylotoxin leading to degranulation[43].

The mechanism of histamine release induced by the autoantibody against Fc€RIα is unusual in that IgE sensitization of basophils is not necessary, whereas histamine release induced by allergens and anti-IgE antibodies is dependent on cell-surface IgE[48].

Antibody subtypes

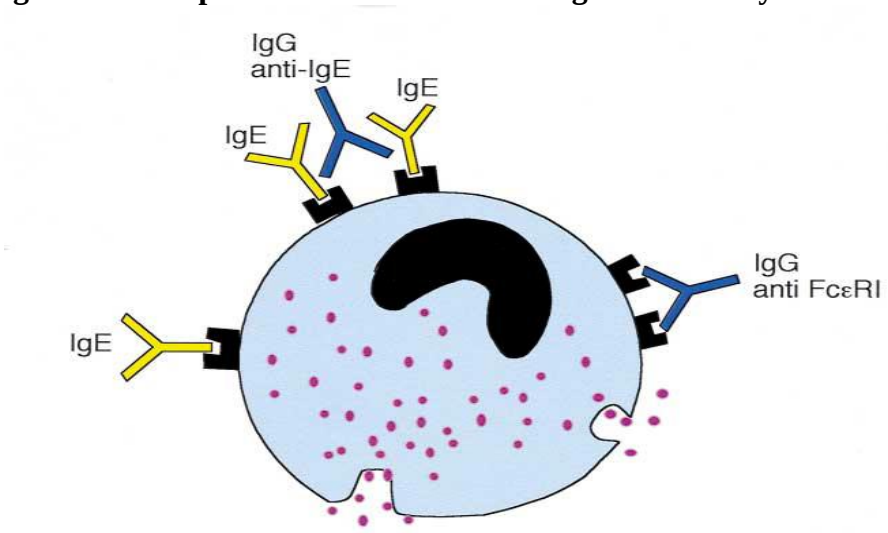
The IgG antibodies detected in chronic autoimmune urticaria are IgG1 and IgG3 subtypes which fix complement[49]. In some patients complement inactivation of serum and complement receptor blockade have shown to inhibit basophil release, suggesting the importance of complement activation and complement receptor signal transduction pathway in them[50]. Mast cells from different sites differ in their expression of complement receptors; for example, mast cells in the skin express C5a receptor, but those in the lung do not[51]. Therefore involvement of

complement might explain the localization of mast cell activation mainly to the skin in CIU.

Positive Immunoblot results for the presence of autoantibodies but negative functional assay results for basophil histamine release were observed in autoimmune disorders, such as dermatomyositis, systemic lupus erythematosus, bullous pemphigoid and pemphigus vulgaris[52]. The antibodies detected in these disorders were of IgG2 and IgG4 subclasses. They are not complement fixing and are not functional and hence unlikely of pathogenic importance.

In some patients some unidentified non IgG factors are responsible for mast cell activation. This group of patients react positively to autologous serum skin test but absence of antibodies in antibody detection assays.

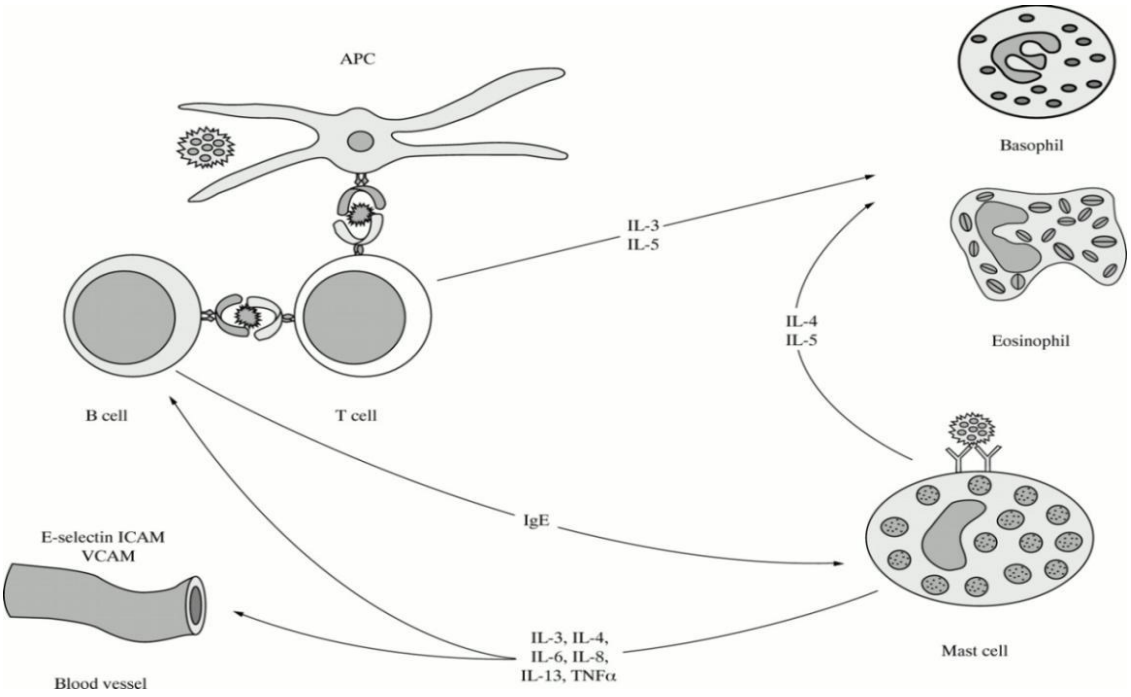
Diagrammatic representation of mast cell degranulation by autoantibodies



Histopathology

The histopathology is usually non specific. There is vascular and lymphatic dilatation in the dermis with edema. Perivascular inflammatory infiltrate surrounding small venules of superficial and deep venular plexus is seen. Infiltrate consists of lymphocytes, neutrophils and eosinophils[53]. Presence of eosinophil varies greatly. Even when eosinophils are not evident, major basic protein can be identified in majority of lesions indicating prior eosinophil degranulation[54]. When the histology of autoimmune and idiopathic chronic urticaria was compared, the autoimmune subgroup had more number of granulocytes within the infiltrate, whereas other infiltrating cells were similar. Increased cytokine levels in the autoimmune group and greater tryptase positivity in the autoantibody negative group was also noted[40]. On electron microscopy, dermal mast cells show signs of degranulation .The perivascular infiltrate consisted predominantly of helper T lymphocytes expressing mRNA for interleukin IL4, IL5 and INFϒ.

DIAGRAMATIC REPRESENTATION OF PATHOGENESIS OF AUTOIMMUNE URTICARIA



Clinical features

Itchy erythematous macules develop into weals consisting of erythematous edematous raised areas of skin often with a surrounding red flare. They occur anywhere on the body, including scalp, palms and soles in variable numbers and sizes ranging from millimeters to lesions covering large areas and in various shapes including rounded, annular, serpiginous and bizarre patterns due to confluence of adjacent lesions.

Weals generally last a few hours and resolve within 24 hours passing through a macular erythematous phase, leaving the skin with normal appearance. The lesions are very itchy especially at night[2, 55].

Patients tend to rub rather than scratch, so excoriation marks are unusual. Occasional bruising may be present.

There may be associated angioedema. These deep swellings which may be the same colour as normal skin occur most frequently on the face, eyelids and lips. Any part of the body may be affected such as ears, neck, hands, feet and genitalia. Mucosal swellings may also occur inside the oral cavity on the buccal mucosa, tongue and pharynx but laryngeal involvement is rare. Angioedema may be preceded by an itching or tingling sensation. Lesions may last for several days[12].

Urticaria may be preceded by vomiting and can be associated with systemic symptoms of malaise, loss of concentration, low mood, feeling hot and cold, headache, abdominal pain, diarrhoea, arthralgia, dizziness and syncope[56]. In most severe acute form it can present with anaphylaxis. Chronic autoimmune urticaria in some studies have shown to have prolonged duration, severe disease manifestation and increased association with systemic symptoms[57].

Associations

Chronic autoimmune urticaria is associated with antithyroid antibodies in 27% of patients[58]. Also it is found to be associated with other auto immune diseases like vitiligo, rheumatoid arthritis, pernicious anemia,

and insulin dependent diabetes mellitus. Family history of urticaria or other autoimmune diseases in the family can be associated[59].

Differential Diagnosis

Urticarial weals are distinguished by their evanescent nature and normal overlying epidermis. Rarely, some diseases can cause difficulty like papular urticaria, erythema multiforme, prebullous eruptions, drug eruptions, Still's disease, mastocytosis, sweet's syndrome, acute contact dermatitis, urticarial vasculitis, lymph edema and collagen vascular diseases like dermatomyositis[12,60]. But these conditions last longer than 24-48 hours.

Detailed clinical history is necessary to differentiate chronic autoimmune urticaria from other causes of urticaria. Information regarding onset, duration, course of the disease, location, number, size and shape of weals are important. Weals lasting for more than 24-48 hours associated with pain suggest possibility of urticarial vasculitis or delayed pressure urticaria, but can also occur in ordinary urticaria. Biopsy of the lesion can be helpful in these cases. Possible precipitating or aggravating factors like heat, cold, localized pressure, friction, sunlight, acute infections, drugs and food should be ruled out.

Investigations

Blood tests - Complete blood count

ESR

Skin biopsy (if vasculitis is suspected).

Stool examination for parasites (if infection suspected).

ENT, Dental evaluation to rule out focal sepsis.

Testing for helicobacter pylori (if symptoms of peptic ulcer is present).

Thyroid function test.

Autologous Serum Skin Test (ASST).

Autologous Plasma Skin Test (APST).

Basophil histamine release assay.

Detection of autoantibodies by ELISA / Immunoblot assay.

IgE levels[12, 61, 62, 63].

Chronic idiopathic urticaria patients are classified under five subsets by Ruth et al in one study based on the differences in immunoreactivity and histamine releasing activity[14],

1. Patients with positive anti Fc€RIα immunoreactivity and positive histamine releasing activity.

2. Patients with positive anti Fc€RIα immunoreactivity but negative histamine releasing activity.

3. Patients with anti IgE antibodies.

4. Serum with ability to release histamine from mast cells but not from basophils.

5. No identifiable factors.

All patients cannot be classified precisely into one of these groups. Patients with both anti Fc€RIα and anti IgE antibodies have been placed in group 3 in this study[14]. This classification of chronic idiopathic urticaria patients helps in identification of basic etiology behind the disease. It cannot be carried out in all settings as it is expensive and requires expertise to perform.

Identification of patients with autoimmune etiology is important in treatment of this subgroup who are at times resistant to conventional therapies where some new treatment options to suppress this autoimmunity can be tried. Autoimmunity can be detected using following tests.

Autologous Serum Skin Tests (ASST)

Autologous serum skin test is a simple in vivo clinical test for detecting presence of circulating functional autoantibodies to Fc€RIα and

or to IgE[5]. Although basophil histamine release assay is the "gold standard" for detecting these functional antibodies, it requires expertise and fresh basophils from healthy donors. Hence ASST can be used as a simple screening test for detecting autoimmune urticaria.

Pre requisite

ASST is performed after the patients are off anti histamines for atleast 3 days (7 days for long acting anti histamines and 2 weeks for doxepin), cortico steroids and other immuno suppressive agents (for 6 weeks to 3 months). The test is not performed over the areas involved by weals in the last 24 hours [5, 64].

Procedure

2ml of patient's venous blood is taken. Blood is allowed to clot under room temperature. Serum is separated by centrifuging blood at 2000rpm for 10 min. 0.1 ml of this separated serum, 0.1ml of normal saline, 0.1ml of histamine (10 micro gram/ml) are injected intradermally at least 5cm apart on the volar aspect of the forearm. Insulin syringe that has 1ml marked as 40 units is used for injection. Every time a separate syringe is used for each injection.

After 30 minutes the weal formed at each injection site is measured at 2 perpendicular diameters (d1&d2) and average is calculated D = d1+d2/2. Positive ASST is one with serum induced weal which has a diameter of ≥ 1.5mm as compared to the saline induced weal at 30 minutes[5].

The reported prevalence of ASST positivity in patients of chronic urticaria ranges from 25-60% in various studies[65,55]. Sera of only half of ASST positive patients are able to release histamine from basophils invitro. Negative ASST patients serum also has shown to contain antibodies. Studies have been done to explain the variations. The various scenarios are:

1. ASST positive but negative antibodies on invitro tests

Some patients react positively to intradermal ASST but show negative results for anti IgE and or anti Fc€RIα antibody detection[66]. When sera of some ASST positive patients were depleted of IgG and then tested again, wheal and flare reactions have been observed. In these patients studies have identified the presence of non Ig histamine releasing factors (HRF) which release histamine from skin mastocytes invivo but fail to degranulate basophils or mast cells invitro. This has put forward the hypothesis that non Ig HRFs may exert a direct activity on cutaneous microvasculature bypassing the cells[67].

2. ASST positive but negative invitro basophil histamine release

Patients with positive ASST and with presence of antibodies have shown negative results with basophil histamine release assays[68]. Sabroe et al in one study indicates that the cause for unresponsiveness of basophils might be due to the autoantibodies recognizing epitopes on recombinant Fc€RIα used in immunoassays that are inaccessible on cell-surface receptors[14]. Other possibility for the false positive ASSTs may be due to the generation of bradykinin during clotting process and cleavage of C5 to C5a by plasma proteases like tryptases secreted by neutrophils[2]. Basophil histamine release by some sera is inhibited when cell-surface Fc€RIα is saturated with IgE, as binding site for the anti Fc€RIα autoantibodies are at or close to the IgE binding site. Dermal mast cells, however, rarely seem to be fully sensitized with IgE. This can be minimized by using IgE stripped basophils for the test[14, 48].

3. ASST negative but positive antibody detection

This can be seen in many diseases in which antibodies are of IgG2 and IgG4 subclass and are non functional detected by their inability to release histamine from basophils[53.]

ASST correlates with severity of urticaria. During remissions ASST has been found to be negative in previously positive patients[9]. The sensitivity of ASST is 70% and specificity is 80%[63].

Limitations

Positive ASST has been demonstrated to be associated with presence of H.pylori antibodies and patients with intolerance to non steroidal anti inflammatory drugs. It needs expertise for reproducibility and stopping antihistamines prior to the test in severe cases may be difficult. Testing over the site of recent weals may alter the results and the results may sometimes need to be correlated with basophil histamine release test.

Autologous Plasma Skin Test (APST)

APST is done by intradermal injection of autologous plasma instead of serum. This is done to prevent release of bradykinin due to clotting of blood in ASST. APST is done by adding anticoagulants other than heparin as heparin can prevent degranulation of mast cells and basophils. Studies comparing ASST and APST have shown that 98% of ASST positive patients showed positive results with APST. 70% of ASST negative patients have shown positive result with APST denoting an increased specificity of APST. The APST positivity and measurement of prothrombin fragments indicate the role of thrombin in chronic urticaria[69].Thrombin increases vascular permeability, causing edema and is able to trigger mast cell degranulation. In some mast cell population, thrombin response was equipotent with Fc€RIα mediated activation.

Diagnosis of Autoimmune Urticaria: In-Vitro Tests

Histamine release assay

This is the gold standard test for the detection of autoimmune urticaria. It detects the functional activity of the serum. This is done by incubating patient serum with basophils or dermal mast cells and detection and measurement of histamine or other mediators released[34].

Basophils are obtained from healthy donors and dermal mast cells are obtained from skin removed during circumcision[70]. Alternatively, rat basophil leukemia cell line transfected with genes to code for Fc€RIα can be used[71]. Basophils used should have both IgE sensitized cells and IgE striped cells (done by lactic acid) for detection of anti IgE and anti- Fc€RIα autoantibodies respectively[34, 72].

Antibody detection

Anti IgE and anti- Fc€RIα auto antibodies can be detected using following tests[71]

1. ELISA
2. Western blot
3. Immunoblotting

Subclass identification of IgG autoantibodies helps in detecting functional and non functional activity.

IgE levels

Elevated IgE levels are detected in chronic urticaria patients. Association has been noted between increased total IgE levels with severe chronic urticaria, positive ASST and antithyroid antibodies[72].

Management

Initial treatment of chronic urticaria is same regardless of patient having autoimmune etiology or not. However Patients with chronic autoimmune urticaria are poor responders to conventional antihistamine therapy. In these patients other therapeutic options can be tried. But antihistamines are the first line drugs to be used.

Antihistamines

Antihistamines are the main stay of treatment in all forms of urticaria, as histamine is the main mediator of urticaria[4]. First generation Classical H1 antihistamines like chlorpheniramine, hydroxyzine, diphenhydramine, etc have side effects including sedation, anti cholinergic effects and paradoxical excitation in children[73]. Newer H1 antihistamines include fexofenadine, loratadine, desloratadine, cetirizine, levocetrizine,

ebastine, mizolastine, olapatadine, rupatadine, etc. Second-generation antihistamines should be considered as the first-line symptomatic treatment for urticaria because of their good safety and tolerability profile. Main advantage is low sedation and minimal anti cholinergic side effects[74]. Higher doses of second generation antihistamines are effective in severe chronic urticaria. Studies using even up to fourfold higher than recommended doses of desloratadine, fexofenadine, levocetrizine and rupatadine can be used[75, 76]. Two long-term studies in healthy volunteers have demonstrated that fexofenadine, at doses up to 240 mg once daily for up to 12 months, is safe and well tolerated[77]. No dose-related increases in corrected QT interval (QTc) were found with fexofenadine doses up to 800 mg once daily or 690 mg twice daily for 28 days[74]. Fexofenadine does not lead to sedation even at supratherapeutic doses. Cetirizine causes sedation at therapeutic and supratherapeutic dose, whereas levocetrizine and desloratadine cause sedation at supratherapeutic dose[78]

In some cases combination of H1 antihistamine with H2 antihistamine may be more effective than H1 antihistamines alone[79]. Use of ranitidine 150mg twice a day is preferable to cimetidine which has more antiandrogenic side effects and potential drug interactions.

Antihistamines cross the placenta. There is no reliable evidence that they are teratogenic but they should be avoided in pregnancy particularly in the first trimester. If not possible, chlorpheniramine appears to be least risky to use[12, 80]. Certain antihistamines have been proposed as preferred for particular subtypes of chronic urticaria, such as hydroxyzine for cholinergic urticaria or cyproheptadine for cold induced urticaria[81].

Although antihistamines are very effective in controlling the symptoms, it is being increasingly realized that these drugs act only at the peripheral level and mask the symptoms. They do not alter the course of the disease that is why in chronic urticaria the symptoms tend to reappear whenever the drug is withdrawn.

Leucotriene receptor antagonists

Zafirlukast (20 mg twice daily) and montelukast (10 mg once daily) are effective in treatment of chronic urticaria especially in cases which were aggravated by the NSAIDs and food additives. Zileuton, a 5-lipooxygenase inhibitor, which inhibits Leucotriene generation is also effective[82].

Tricyclic antidepressants

Doxepin is a tricyclic antidepressant with very potent H-1 and H-2 antihistaminic properties. Doxepin is more effective than diphenhydramine

at a dosage of 10 mg thrice daily. Doxepin should not be advised after a recent myocardial infarction or for patients with severe liver disease. It is best to start at a low dose of 10 mg daily at night, which can be increased to 20 or 30 mg a day[83, 84].

Corticosteroids

Oral corticosteroids are effective in severe urticaria not controlled with antihistamines. Dose of 0.5–1mg of prednisolone/kg/day can be given[7]. Steroids do not inhibit mast cell degranulation but affect the function and cytokine production by inflammatory cells[85]. Long term dosage should be avoided because of risk of side effects[86]. Short term alternate day regimen can be used for severe, persistent episodes[87].

Autologous serum therapy (AST)

AST is an effective treatment option for patients with chronic autoimmune urticaria identified by ASST. It is given by injecting patients own serum as a weekly once intramuscular injection for a period of 9 consecutive weeks. 5 ml of patient blood is drawn and centrifuged at 2000rpm for 10 minutes. 2ml of the separated serum is injected as deep intramuscular injection[5]. Previously whole blood was drawn and injected (autohemo therapy) [88, 89]. But this was very painful and the injections should be given immediately .This was replaced by serum therapy as the

antibodies are concentrated in the serum than the cellular components of blood.

Studies conducted on AST in chronic autoimmune urticaria patients have shown variable results. The exact mechanism by which AST acts is not known, one study indicated 60% patients had significant improvement in their signs and symptoms[5]. Relapse of symptoms have been noted during follow-up which responded with booster dose of AST. Interesting finding in the study by Bajaj et al. is that, the ASST-negative group responded almost equally well to AST as the ASST-positive group. Existence of a subset of patients with autoimmune urticaria who are ASST-negative may be considered as a possibility for the response[87].

AST is a cheap, effective and potentially curative modality in some patients with recalcitrant chronic urticaria.

Calcium channel blockers

Limited evidence shows that calcium channel blocker, nifedipine at dosage of 10 mg twice daily to 20 mg three times daily used in combination with antihistamines, has added effect in patients who have not responded to antihistamines alone[90].

Immuno modulators

Cyclosporine

Various studies have been conducted for the effect of cyclosporine in chronic urticaria. Dose use ranged from 3-6 mg/kg/day[91, 92]. In a randomized doudle blinded study, 4mg/kg of cyclosporine administered over 4 weeks resulted in a significant response[93]. In other randomized study dose of 5mg/kg slowly tapered to 3mg/kg was given over a period of 16 weeks[94]. It is useful in patients with severe chronic urticaria. It spares the need for corticosteroids. In autoimmune urticaria patients, significant reduction of invitro histamine-releasing activity and reduction in reaction size of the autologous serum skin test was seen after cyclosporine treatment[95]. Mechanism of action is by inhibition of basophil and mast cell degranulation by interfering with intracellular signaling in cells following recptor cross linking[87].

Methotrexate

There are only few case reports of methotrexate use in chronic urticaria. No randomized control studies are available for its use in chronic autoimmune urticaria. Gach et al reported its use in two patients with severe chronic idiopathic urticaria[96]. Dose of 2.5 mg orally twice daily for two days in a week was tried in a study on chronic autoimmune urticaria

patients who were refractory to treatment with antihistamines. Treatment was given for two months along with antihistamines. After a course of treatment patients were controllable with antihistamines alone[97].

Warfarin

Dose of 2-5 mg/day of warfarin for 2-5 months has been shown to benefit patients not controlled with antihistamines and requiring daily dose of steroids. A double blind placebo controlled study conducted by Parslew et al on chronic idiopathic urticaria patients has shown significant benefit in reduction of pruritus and angioedema with warfarin[98]. The mechanism of warfarin action is by suppression of thrombin, a potent mast cell degranulator and proteins in the coagulation cascade[99]. Involvement of the extrinsic coagulation pathway in chronic idiopathic urticaria has been demonstrated, providing the rationale for anticoagulant use[100]. Repeated prothrombin time monitoring is important to avoid major adverse effects like bleeding. PT INR should be maintained between 2 and 2.5. In chronic urticaria patients with and co existing diseases requiring long term warfarin like deep vein thrombosis, atrial fibrillation and recurrent pulmonary embolism this therapy may be considered[101].

Omalizumab

Omalizumab is a recombinant humanized monoclonal antibody that selectively binds to IgE. In chronic autoimmune urticaria patients it acts by inhibiting the binding of IgE to the high affinity IgE receptor (Fc€RIα) on the surface of mast cells and basophils and by sufficient reduction in Fc€RIα expression on the surface of these cells. It prevents IgG antibody-mediated cross-linking of adjacent α subunits causing mediator release[102,103,104].

Dosing is based on weight and is administered via subcutaneous injection every 2-4 weeks[103]. Dose tried is 300mg once a month. The safety profile of omalizumab is favorable with injection site reaction being the most commonly reported adverse event[102].

Intravenous immunoglobulin

Intravenous immunoglobulin dose of 0.4gm /kg/day for 5 days was effective in relieving symptoms and achieving ASST negativity in chronic autoimmune urticaria patients. Long term remissions were reported[46]. Permanent remission was noted in patients attaining ASST negativity within 6 months of therapy. High dose of 2g/kg over 24 hours was also used successfully[105]. Possible mechanism of action is by the presence of anti idiotype antibodies suppressing the IgE autoantibodies[106].

Cyclophosphamide

Use of cyclophosphamide was reported by Bernstein et al in woman with severe chronic autoimmune urticaria. Initial dose given was 500 mg every 2 weeks, increased by 100 mg every 2 weeks till a maximum of 1500 mg was reached, which was continued every 4 weeks. Complete clinical remission was noted in 7 months. Repeat ASST was negative. Possible mechanism of action is that, cyclophosphamide may have eradicated B-cell clones that were producing autoantibodies to the high-affinity IgE receptor. This study is the only known report of the use of this agent in chronic urticaria[107].

Plasmapheresis

Plasmapheresis was found to be beneficial in a study conducted on eight patients with chronic autoimmune urticaria. It may act by eliminating the functional autoantibodies from body. A decrease in the size of the skin test reaction to autologous serum after plasmapheresis was noted[15].

Dapsone

Dose of 50 mg/day of dapsone was used in a study on a patient with chronic urticaria not controlled with antihistamines and required large doses of steroid. The mechanism responsible for clearing may be by down

regulation of leukotriene B_4 (LTB_4) and/or interference with CD11b, which has shown to play a role in chronic urticaria[108].

Others

Other modalities tried include,

Sodium cromoglycate in a dose of 200 mg four times daily was used in a study. It inhibits release of mediators from mast cell, by interfering with calcium transport across the mast cell membrane[109]. Patients with chronic urticaria and thyroid autoimmunity may improve with thyroxine treatment[110].

Other drugs found to be helpful in small studies or case reports, include, colchicine, hydroxychloroquine[111], oral tacrolimus[112], mycophenolate mofetil[113], PUVA[114] and sulphasalazine[115].

Future possibilities

More selective immunotherapies are possibilities. The extracellular part of the α subunit of Fc€RIα or shorter peptide sequences containing the autoantibody epitopes could be used to bind to circulating Fc€RIα autoantibodies, thereby inhibiting their attachment to receptors on mast cells or basophils[4].

Aim of the study

AIM OF THE STUDY

1. To study the prevalence of chronic autoimmune urticaria among patients with chronic idiopathic urticaria using autologous serum skin test.

2. To study the epidemiological pattern of chronic autoimmune urticaria.

3. To evaluate the efficacy of autologous serum therapy in patients with chronic autoimmune urticaria.

Materials and methods

MATERIALS AND METHODS

Study design: Prospective, Interventional study.

Study period : Oct 2009 to Sep 2011.

Study population : 200 consecutive patients with chronic idiopathic urticaria attending our dermatology OP were selected for the study

The study was approved by the Institutional Ethical Committee, Rajiv Gandhi Government General Hospital & Madras Medical College, Chennai. All patients signed a written informed consent document.

Diagnosis of chronic urticaria was done based on the history and clinical examination. History of drug intake, aggravating food, urticaria following infections, allergy to house dust, pollen, animal danders, history of episodes following exposure to cold/heat/pressure/sunlight/exercise, history of dental/ortho implants was taken to rule out other causes of urticaria.

Details from the history included in the study

1. Age
2. Sex
3. Total duration of the disease

4. Frequency of episodes
5. Number of weals
6. Severity of pruritus
7. Duration of each episode
8. Frequency of AHT use
9. Size of weals
10. Associated atopy / angiodema

Scoring

Following scoring was done based on the history to grade the severity of the disease.

1. Number of Weals score

0 – none

1 - ≤ 10

2 – 11 to 50

3 - >50

2. Pruritus score

0 – Absent

1 – Present but not disturbing

2 – Disturbing but not hampering sleep / day time activity

3 – Hampering day time activity / sleep

3. Frequency of weal score

0 – none

1 - < once / once a week

2 – 2 to 3 times a week

3 – Almost daily / daily

4. Duration of weal score

0 – none

1 - < 1 hour

2 – 1 to 12 hours

3 - >12 hours

5. Score for antihistamines use

0 – None

1 - < once / once a week

2 – 2 to 3 times a week

3 – Almost daily / daily

6. Size of weal score

0 – None

1 - < 1cm

2 – 1 to 3 cm

3 - > 3 cm

Total severity score (TSS)

TSS is calculated by adding the scores for number of weals, pruritus, frequency of weal, duration of weal, antihistamine use and size of weal.

Maximum TSS is 18. Based on the TSS value patient's disease severity was graded as follows:

TSS SCORE	GRADE
0	clear
1 – 6	mild
7 – 12	moderate
13 - 18	severe

Inclusion criteria for ASST

Patients with chronic idiopathic Urticaria.

Duration of weals for more than six weeks.

Patients more than 12 years of age.

Willingness for the test.

Exclusion criteria for ASST

Urticaria due to medications, insect bites, food allergy, following acute or chronic infections or other known etiology(from history).

Physical Urticaria and hereditary angioedema.

Less than 12 years of age.

Pregnant and lactating women.

Inclusion criteria for AST

Patients with positive ASST.

Willingness for weekly injections.

Willingness for follow up.

Exclusion criteria for AST

Patients with negative ASST.

Pregnant and lactating women.

Age less than 12 years

Investigations performed to rule out other causes are

Complete blood count

Urine routine

Liver function test

Renal function test

Stool examination

ENT and dental examination to rule out focal sepsis

Materials required

Sterile 2 ml and 5 ml syringes

Sterile test tubes

Centrifuge

Insulin syringes

Normal saline

Prerequisites for ASST

Patient should be free from drugs that can cause false negative reactions. If patients had taken the following drugs, ASST was done after the wash out period for the drugs.

Drug	**Wash-out period**
Corticosteroids –	
Parenteral (IM, intra-articular)	≥ 90 days
Oral Corticosteroids	≥ 30 days
Topical Corticosteroids	≥ 14 days
Inhaled Corticosteroids	≥ 30 days
Antihistamines - Astemizole	≥ 60 days
Loratidine	≥ 7 days

MATERIALS REQUIRED FOR AUTOLOGOUS SREUM SKIN TEST

CENTRIFUGE MACHINE

Drug	**Wash-out period**
Certizine,Terfenadine, Fexofenadine Hydroxyzine, Ebastine, Azatadine	≥ 72 hours ≥ 72 hours
H2 Antagonists	≥ 72 hours
Other H1 antagonists	≥ 24 hours
Cough / Cold preparations	≥ 24 hours
Sedatives & hypnotics	≥ 24 hours
Tricyclic antidepressants	≥ 21 days
Phenothiazines, Benzodiazepines	≥ 21 days
NSAIDS & Narcotic analgesics	≥ 72 hours

Procedure

Method of serum separation

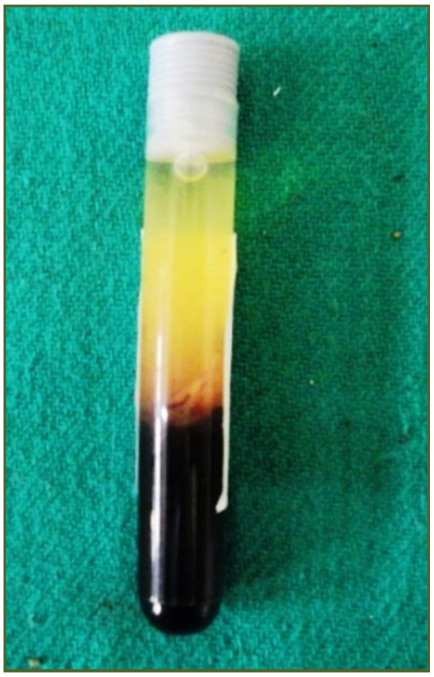
1. Two ml patient's venous blood is taken from antecubital vein.
2. Blood is allowed to undergo clotting at room temperature.
3. Serum is separated by centrifugation done at 2000 rpm for 10 minutes.

Test

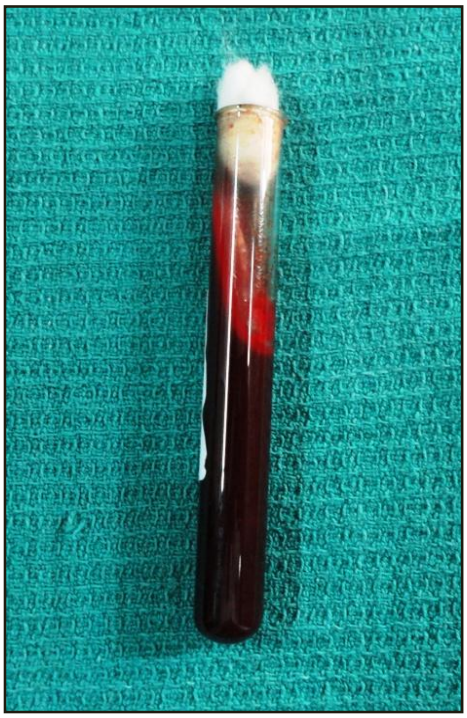
0.1 ml of the seperated serum is injected intradermally using an insulin syringe into the volar aspect of forearm, avoiding the areas of wealing within the past 24 hours.

2 ML BLOOD COLLECTED FOR ASST

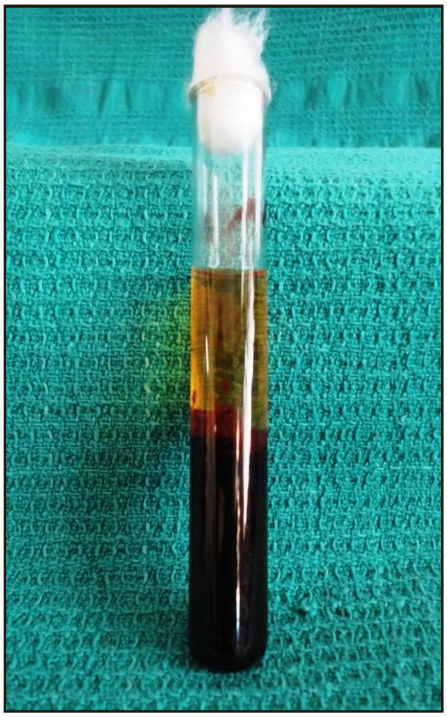
SERUM SEPERATED FROM 2 ML FOR ASST



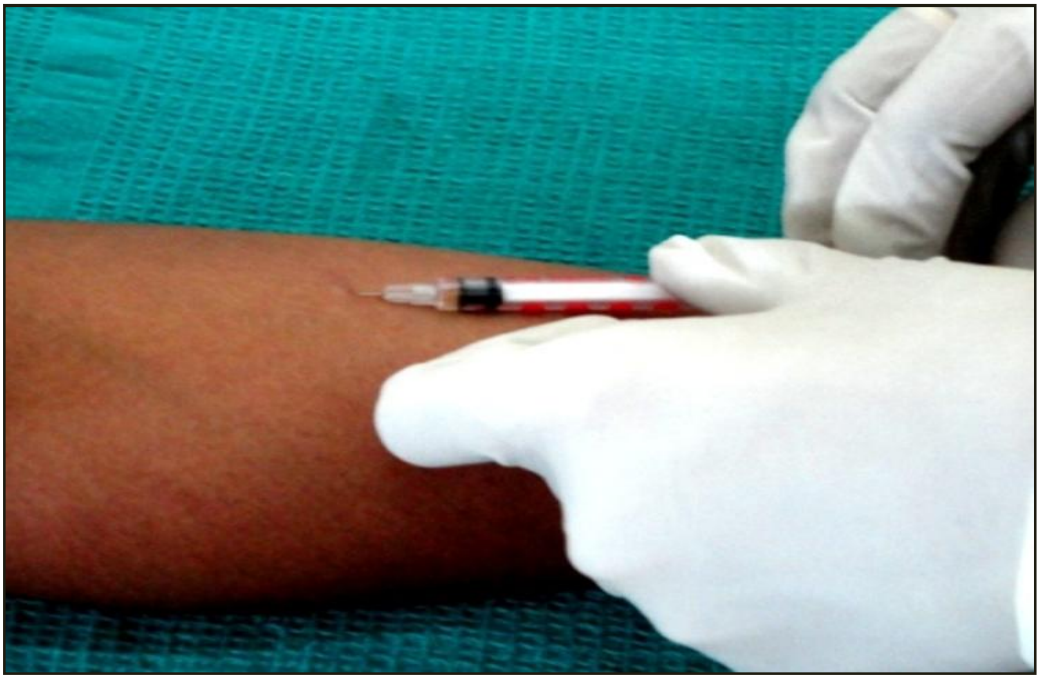
5 ML BLOOD COLLECTED FOR AST



2 ML OF SERUM SEPERATED FOR AST



TECHNIQUE OF AUTOLOGOUS SERUM SKIN TEST



Control

Equal amounts of normal saline is injected intradermally using separate insulin syringe 3 to 5 cm apart in the volar aspect of the same forearm for negative control.

Reading

Wheal and flare response is measured after 30 min.

Vertical diameter (d1), horizontal diameter (d2) are measured and average diameter D is calculated as D = d1+d2/2

Positive test

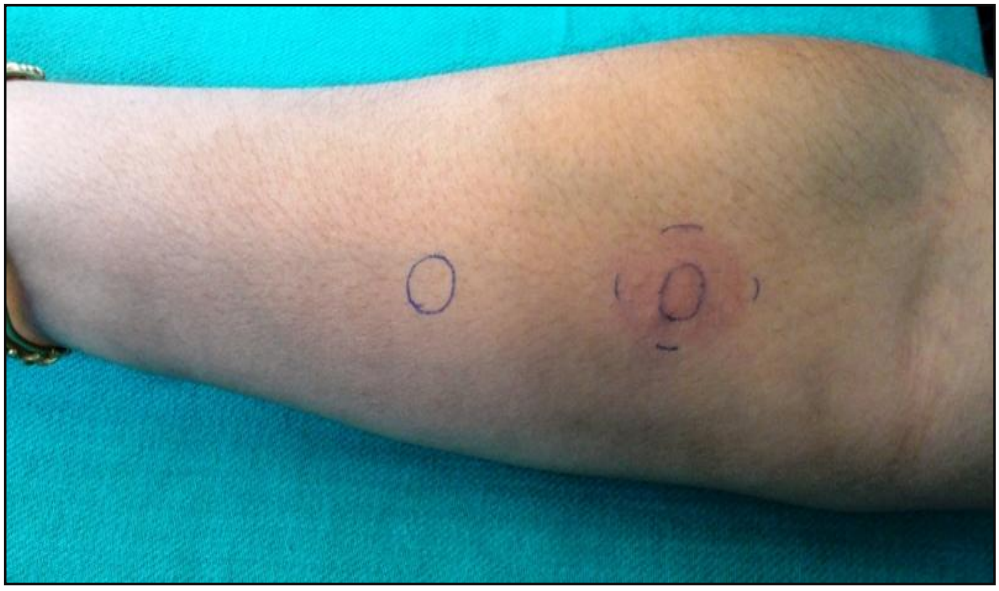
Positive result is defined as the serum induced weal with a diameter of $\geq$ 1.5 mm as compared to the saline induced weal.

Autologous serum therapy

Method of serum separation

1. Five ml of patient's venous blood is taken from antecubital vein.
2. Blood is allowed to undergo clotting at room temperature.
3. Serum is separated by centrifugation at 2000rpm for 10 minutes.

POSITIVE RESULT OF AUTOLOGOUS SERUM SKIN TEST



NEGATIVE RESULT OF AUTOLOGOUS SERUM SKIN TEST

Therapy

2 ml of the separated serum is given as deep intramuscular injection in alternate buttock once a week for a period of 9 consecutive weeks.

Rescue antihistamines were permitted during therapy period when symptoms occur and their frequency of use noted for scoring. All patients were given Tab.cetrizine uniformly, to be used as rescue therapy when need arises.

Follow up

Patients were asked to come for follow up after 3 months of last injection. Repeat scoring is done and TSS is calculated.

Statistical analysis

Statistical analysis was performed using chi-square test, independent t test and paired t test. P values <0.05 was considered significant. Comparison between ASST positive and ASST negative patients was done in relation to

Age

Sex distribution

Duration of disease

Atopy

Angioedema

TSS

CLINICAL PHOTOGRAPH OF URTICATIAL WEALS

URTICARIAL WEALS ON THIGH

ANGIOEDEMA OF HAND

Observations and results

OBSERVATION AND RESULTS

200 consecutive patients of chronic idiopathic urticaria satisfying the study criteria were selected for the study. Of the total 200 patients 85 (42.5%) were males and 115 (57.5%) were females.

Table 1 : sex distribution of chronic urticaria

GENDER	FREQUENCY	PERCENT
Male	85	42.5
Female	115	57.5
Total	200	100.0

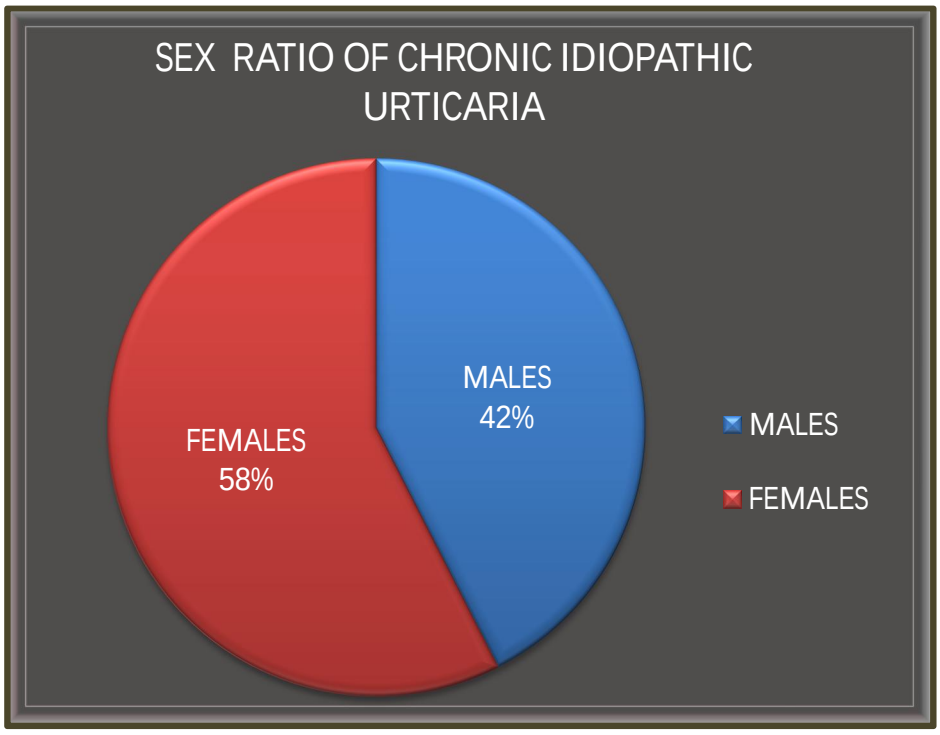
1. PREVALENCE OF AUTOIMMUNE URTICARIA

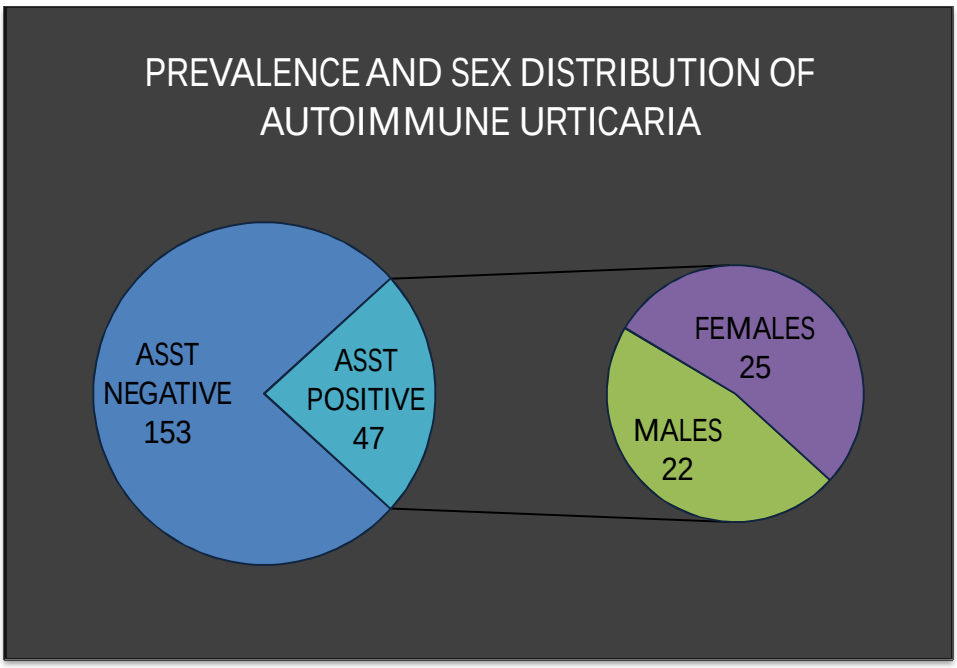
ASST was performed on 200 patients using autologous serum. Of which 153(76.5%) were negative for ASST and 47(23.5%) were positive.

Table 2: Prevalence of chronic autoimmune urticaria

ASST RESULT	FREQUENCY	PERCENT
Negative	153	76.5
Positive	47	23.5
Total	200	100.0

Based on ASST results, patients are grouped into two groups, ASST positive and ASST negative. Age, sex, duration of disease, Total Severity Score (TSS), association with atopy/angioedema was compared between


SEX RATIO OF CHRONIC IDIOPATHIC URTICARIA
MALES 42%
FEMALES 58%
MALES
FEMALES


PREVALENCE AND SEX DISTRIBUTION OF AUTOIMMUNE URTICARIA
ASST NEGATIVE 153
ASST POSITIVE 47
FEMALES 25
MALES 22

the two groups. TSS was calculated based on parameters like frequency of appearance of weals, number of weals during each episode, pruritus score, duration of each episode, AHT use and size of weals.

2. SEX DISTRIBUTION

Table 3 : Comparison of sex distribution

ASST RESULT	GENDER				P Value
	Male		Female		
	NUMBER	%	NUMBER	%	
Negative	63	74	90	78	0.5048
Positive	22	26	25	22	
Total	85	100.0	115	100.0	

Of the total 47 ASST positive patients, 22(47%) were males and 25(53%) were females. Of the total 153 ASST negative patients, 63(41.17%) were males and 90(58.82%) were females. There was no statistical significance in the sex distribution between ASST positive and negative patients(P=0.5048).

3. AGE DISTRIBUTION

Table 4 : Comparison of mean age

ASST RESULT	NO OF PATIENTS	AGE IN YEARS		P VALUE
		MEAN	STD DEVIATION	
Positive	47	36.77	14.55	0.535
Negative	153	35.42	12.48	

Table 5 : Comparison of age distribution

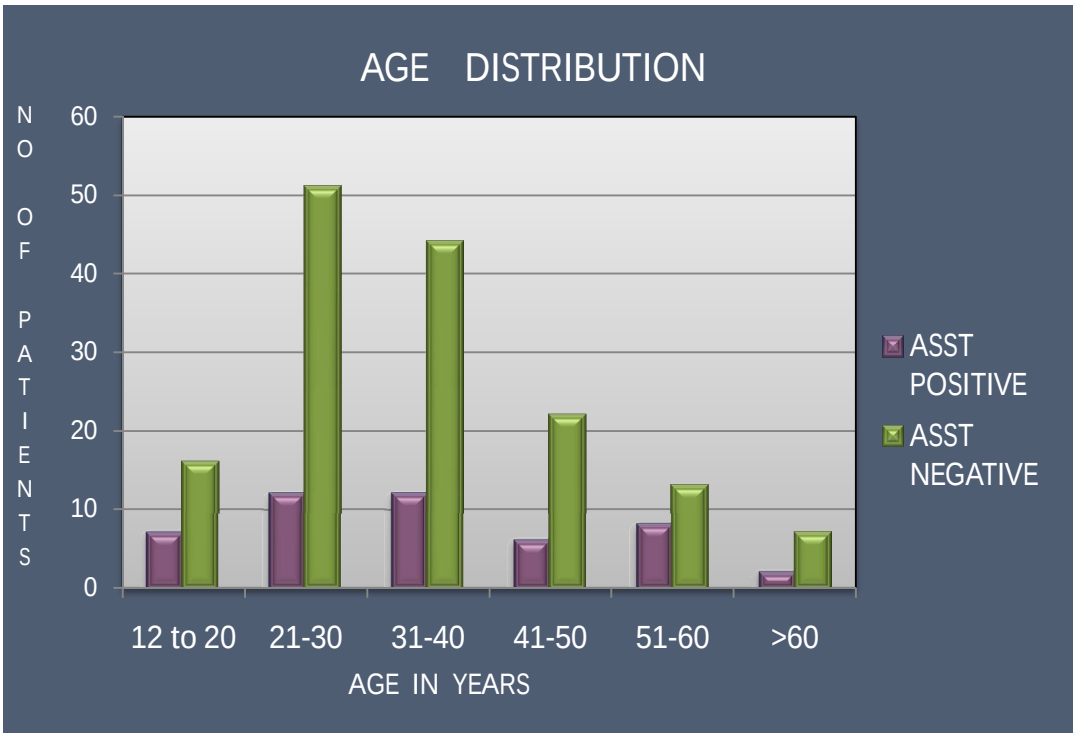
AGE GROUP	ASST POSITIVE	ASST NEGATIVE
12-20	7	16
21-30	12	51
31-40	12	44
41-50	6	22
51-60	8	13
>60	2	7
TOTAL	47	153

Mean age in ASST positive patients was 36.77±15 yrs and in ASST negative patients were 35.42±13 yrs. There was no statistical difference in the age distribution between ASST positive and negative groups (P value is 0.535).

4. DURATION OF DISEASE

Table 6 : Comparison of total duration of disease

TOTAL DURATION	ASST POSITIVE	ASST NEGATIVE
<1 year	24	102
>1 to <3 yrs	9	36
>3 to <5 yrs	7	5
>5 years	7	10


AGE DISTRIBUTION
NO OF PATIENTS
60
50
40
30
20
10
0
12 to 20
21-30
31-40
41-50
51-60
>60
AGE IN YEARS
ASST POSITIVE
ASST NEGATIVE

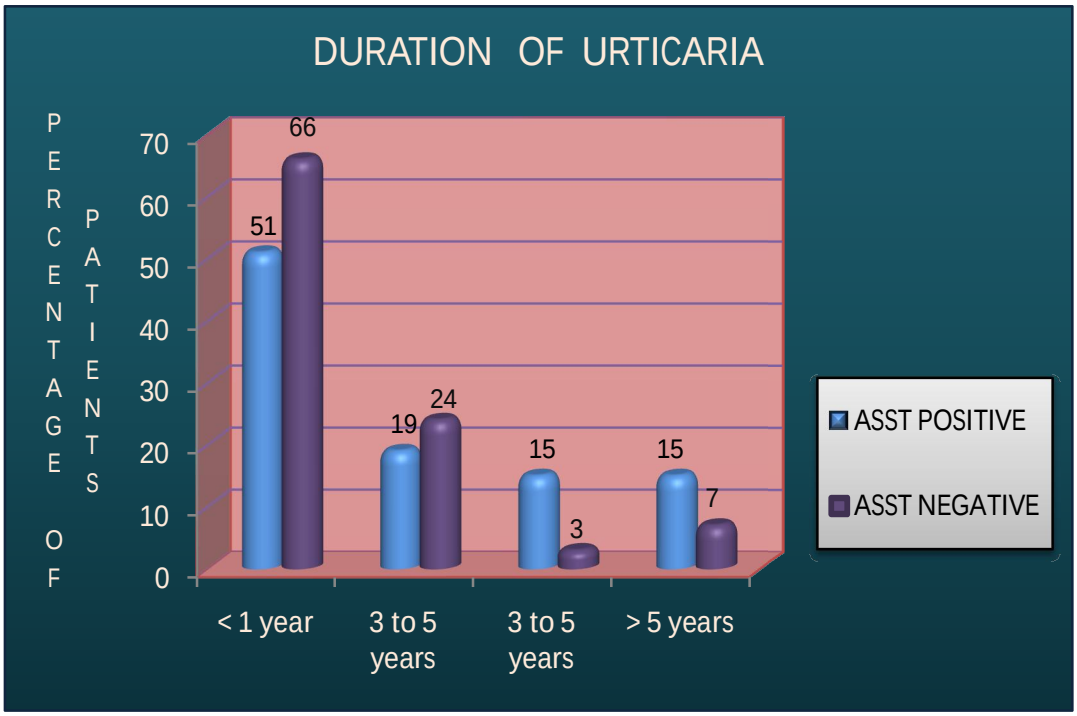

DURATION OF URTICARIA
PERCENTAGE OF PATIENTS
70
60
50
40
30
20
10
0
51
66
19
24
15
3
15
7
< 1 year
3 to 5 years
3 to 5 years
> 5 years
ASST POSITIVE
ASST NEGATIVE

Table 7 : Comparison of mean for total duration of disease

ASST RESULT	NO OF PATIENTS	DURATION IN YEARS		P VALUE
		MEAN	STD DEVIATION	
Positive	47	4.36	2.78	0.142
Negative	153	3.58	3.29	

Mean duration of disease in ASST positive group was 4.36±3 years and in ASST negative group was 3.58±3 years. There was no statistical significance in the age distribution between two groups (P value = 0.142).

5. ATOPY

Table 8 : Comparison of association with atopy

ASST RESULT	ATOPY		P VALUE
	PRESENT	ABSENT	
Negative	42	111	0.852
Positive	14	33	
Total	56	144	

In the ASST positive group out of 47 patients, atopy was present in 14 patients (29.78 %). In the ASST negative group out of 153 patients, atopy was present in 42 patients (27.45 %). There was no statistical significance of association with ASST result and atopy (P=0.852).

6. ANGIODEMA

In the ASST positive group out of 47 patients, angiodema was present in 28 patients (59.57%). In the ASST negative group out of 153

patients, angiodema was present in 52 patients (33.98%). There was statistically significant association with angiodema in the ASST positive group compared to ASST negative group(P = 0.002).

Table 9 : Comparison of association with angioedema

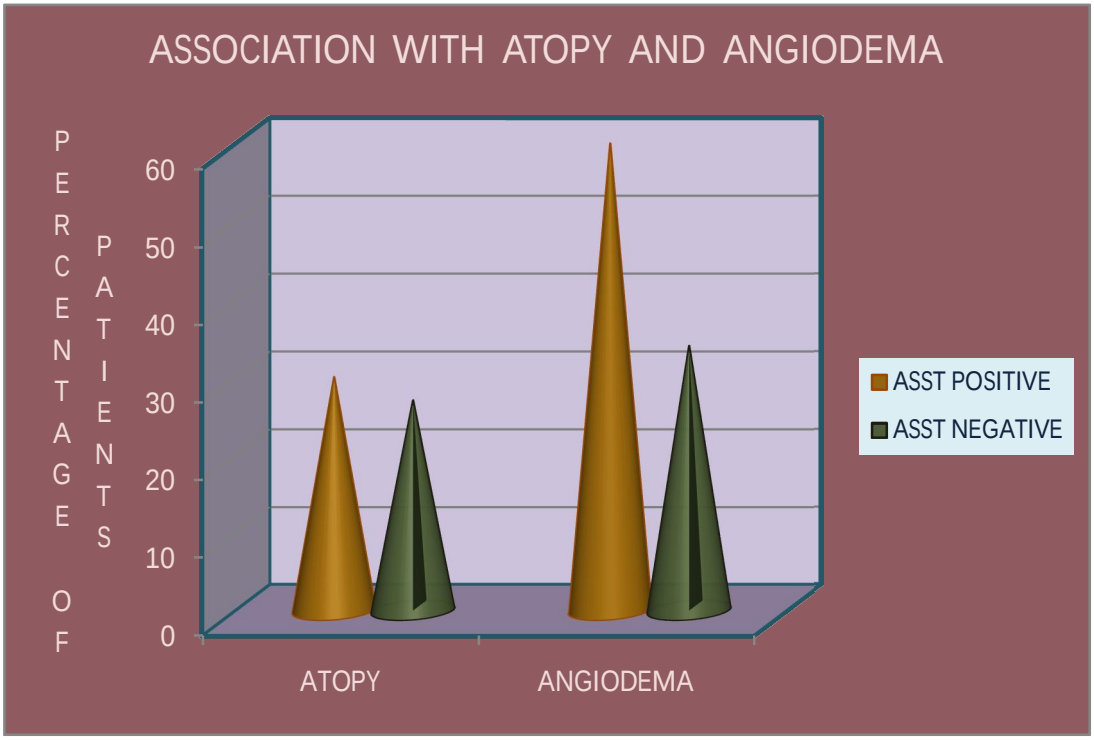
ASST RESULT	ANGIODEMA		P VALUE
	RESENT	ABSENT	
Positive	28	19	0.002
Negative	52	101	
Total	80	120	

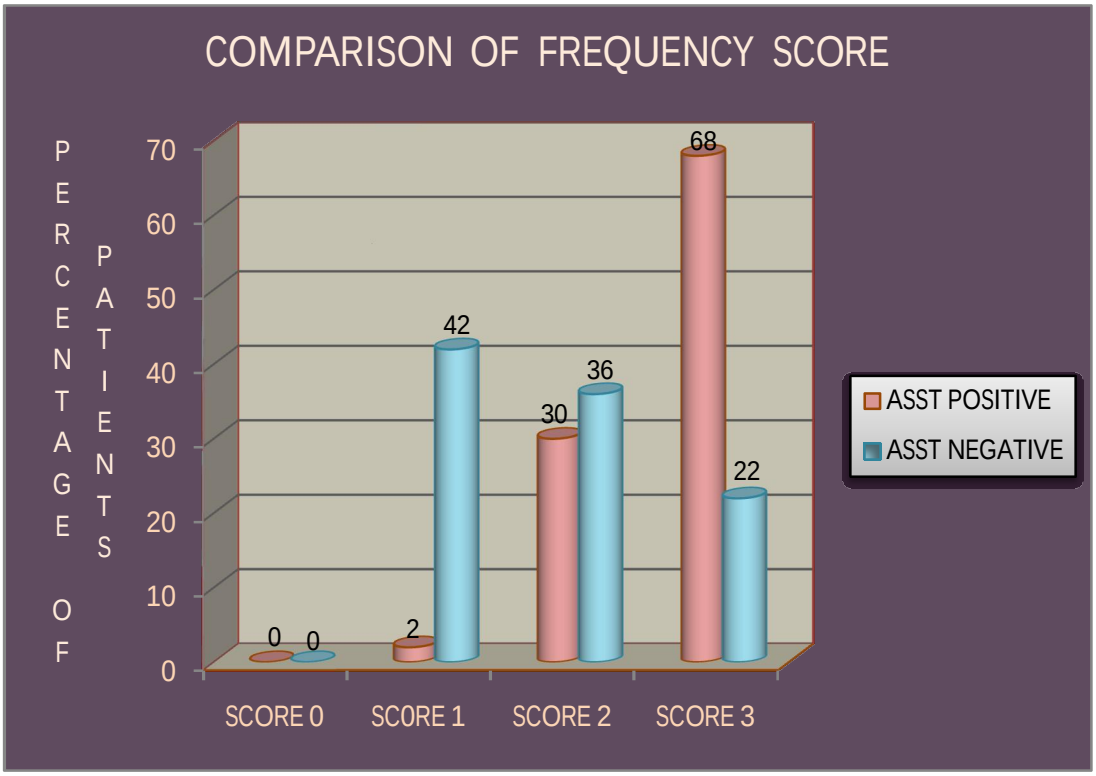
7. FREQUENCY SCORE

In ASST positive group, 1(2.13%) had score 1, 14(29.78%) had score 2 and 32 patients (68.08 %) had score 3. In ASST negative group, 65(42.48%) had score 1, 55(35.94%) had score 2 and 33 patients (21.56 %) had score 3. ASST positive patients had more frequent episodes of urticaria than negative group. There was statistical significance in frequency score between two groups(P<0.001).

Table 10 : Comparison of frequency score

ASST RESULT	FREQUENCY SCORE			P VALUE
	1	2	3	
Positive	1	14	32	<0.001
Negative	65	55	33	
Total	66	69	65	


ASSOCIATION WITH ATOPY AND ANGIODEMA
PERCENTAGE OF PATIENTS
60
50
40
30
20
10
0
ATOPY
ANGIODEMA
ASST POSITIVE
ASST NEGATIVE


COMPARISON OF FREQUENCY SCORE
PERCENTAGE OF PATIENTS
70
60
50
40
30
20
10
0
0
0
2
42
30
36
68
22
SCORE 0
SCORE 1
SCORE 2
SCORE 3
ASST POSITIVE
ASST NEGATIVE

8. NUMBER OF WEALS SCORE

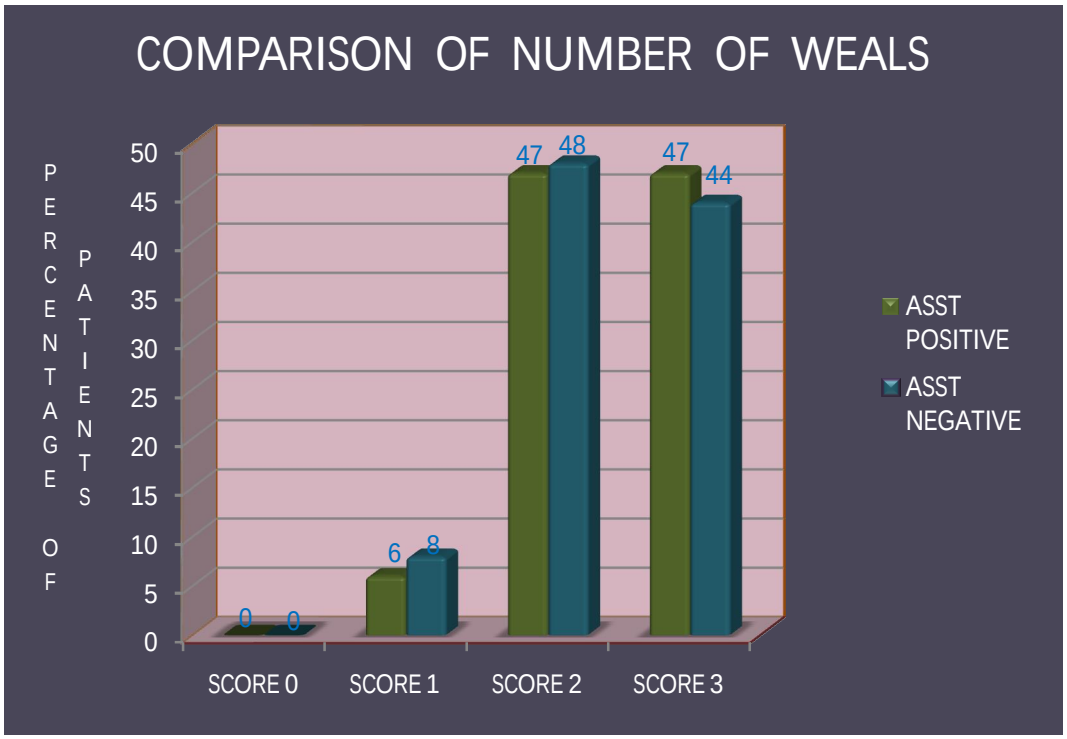
Table 11 : Comparison of score for number of weals

ASST RESULT	NUMBER OF WEALS SCORE			P VALUE
	1	2	3	
Positive	3	22	22	0.926
Negative	12	73	68	
Total	15	95	90	

Out of 47 ASST positive patients, 3(6.34%) patients had score 1, 22(46.8 %) had score 2 and 22(46.8 %) had score 3. Out of 153 ASST negative patients 12(7.84%) had score 1, 73(47.71 %) had score 2 and 68(44.44%) had score 3. There was no statistical significance between two groups (P=0.926).

9. PRURITUS SCORE

In the ASST positive group, 42.55%(20 patients) had score 3, 46.80%(22 patients) had score of 2 and only 10.63%(5 patients) had score 1. In ASST negative group 29.41 %(45 patients) had score 1, 42.48%(65 patients) had score 2 and 28.10% (43 patients)had score 3. The difference in the severity between two groups was statistically significant(P=0.022).


COMPARISON OF NUMBER OF WEALS
PERCENTAGE OF PATIENTS
50
45
40
35
30
25
20
15
10
5
0
0
0
6
8
47
48
47
44
SCORE 0
SCORE 1
SCORE 2
SCORE 3
ASST POSITIVE
ASST NEGATIVE

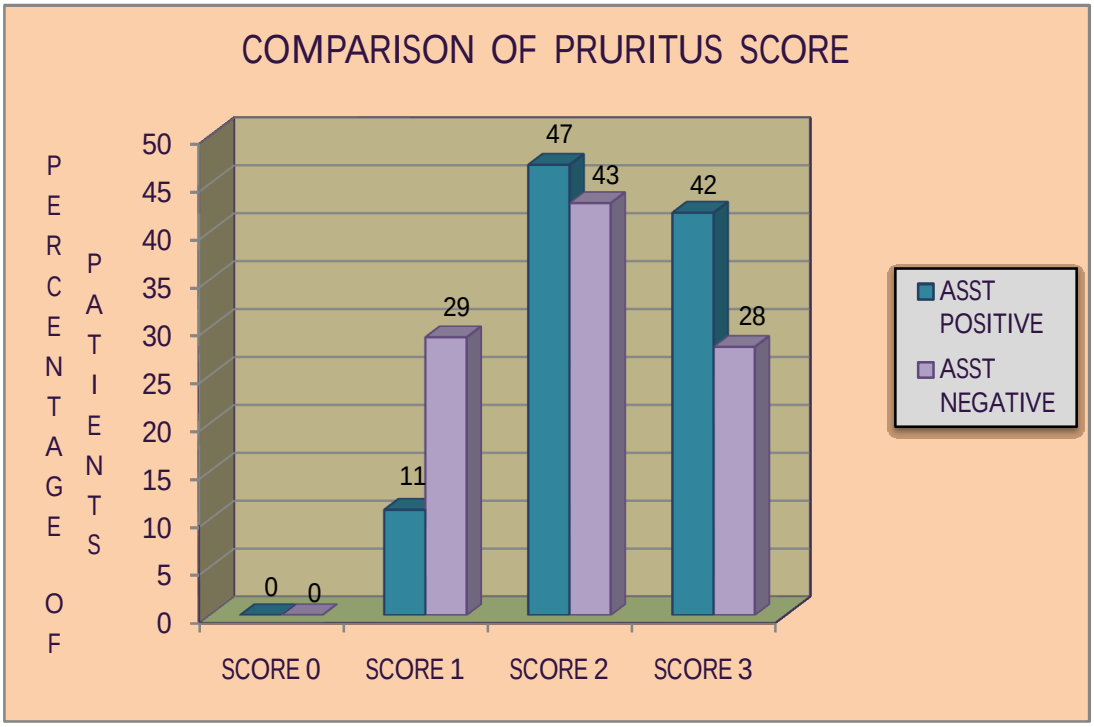

COMPARISON OF PRURITUS SCORE
PERCENTAGE OF PATIENTS
50
45
40
35
30
25
20
15
10
5
0
0
0
11
29
47
43
42
28
SCORE 0
SCORE 1
SCORE 2
SCORE 3
ASST POSITIVE
ASST NEGATIVE

Table 12 : Comparison of pruritus score

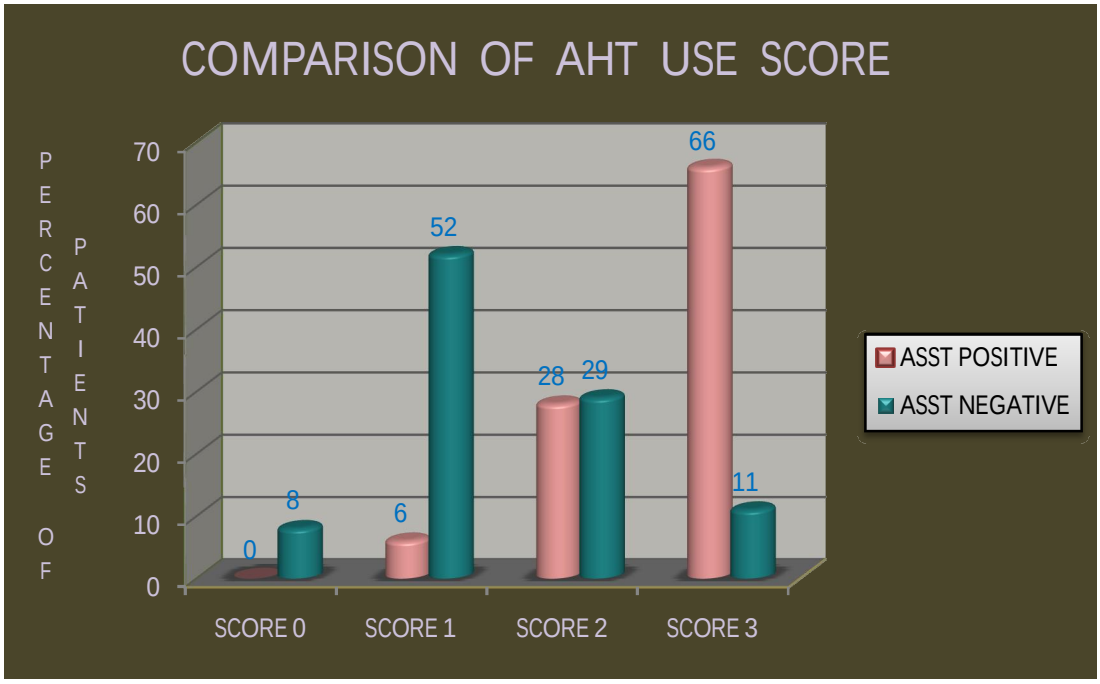
ASST RESULT	PRURITUS SCORE			P VALUE
	1	2	3	
Positive	5	22	20	0.0223
Negative	45	65	43	
Total	50	87	63	

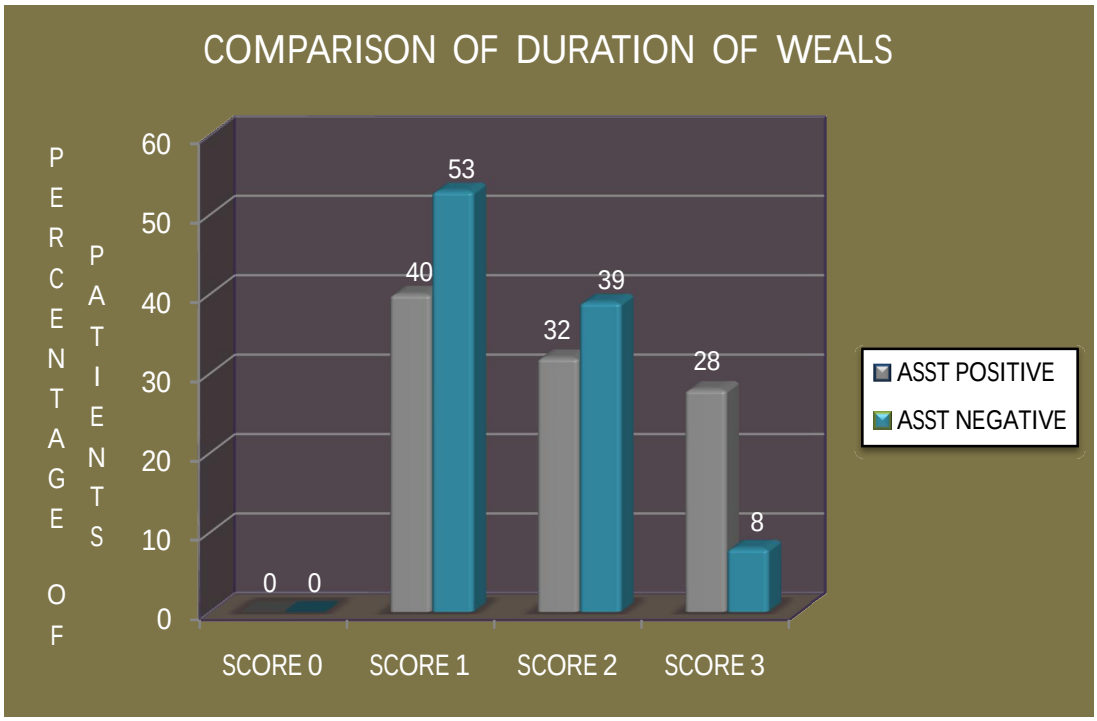
10. AHT USE

In the ASST positive group none of them had score 0, 3(6.38%) patients had score 1, 13(27.66%) had score 2 and 31(65.96%) had score 3. In ASST negative patients 13(8.49 %) patients had score 0, 79(51.63%) had score 1, 44(28.76%) had score 2 and 17(11.11%) had score 3. Score 0 indicated that patients had mild symptoms which was tolerated without any treatment. The difference in the score between two groups was statistically significant ($p<0.001$).

Table 13 : Comparison of score for antihistamine requirement

ASST RESULT	AHT USE SCORE				P VALUE
	0	**1**	**2**	**3**	
Positive	0	3	13	31	<0.001
Negative	13	79	44	17	
Total	13	82	57	48	


COMPARISON OF AHT USE SCORE
PERCENTAGE OF PATIENTS
70
60
50
40
30
20
10
0
0
8
6
52
28
29
66
11
SCORE 0
SCORE 1
SCORE 2
SCORE 3
ASST POSITIVE
ASST NEGATIVE


COMPARISON OF DURATION OF WEALS
PERCENTAGE OF PATIENTS
60
50
40
30
20
10
0
0
0
40
53
32
39
28
8
SCORE 0
SCORE 1
SCORE 2
SCORE 3
ASST POSITIVE
ASST NEGATIVE

11. DURATION OF WEALS

Table 14 : Comparison of weal duration score

ASST RESULT	WEAL DURATION SCORE			P VALUE
	1	2	3	
Positive	19	15	13	0.0015
Negative	82	59	12	
Total	101	74	25	

In ASST positive group 19(40.42%) patients had score 1, 15(31.91%) had score 2 and 13(27.65%) had score 3. In ASST negative group 82(53.59%) patients had score 1, 59(38.56%) had score 2 and 12(7.84%) had score 3. Percentage of patients having severe score was higher in ASST positive group while majority of ASST negative group had score 1.The difference was statistically significant (P=0.0015).

12. WEAL SIZE

In ASST positive patients around 19.14 %(9 patients) had score 1, 63.83 %(30 patients) had score 2 and 17.02 %(8 patients) had score 3. In ASST negative patients 37.91 %(58 patients) had score 1, 49.67% (76 patients) had score 2 and 12.42% (19 patients) had score 3. Percentage of patients having severe score was higher in ASST positive group. There was no statistical significance between two groups in the weal size score (P=0.057).

Table 15 : Comparison of score for weal size

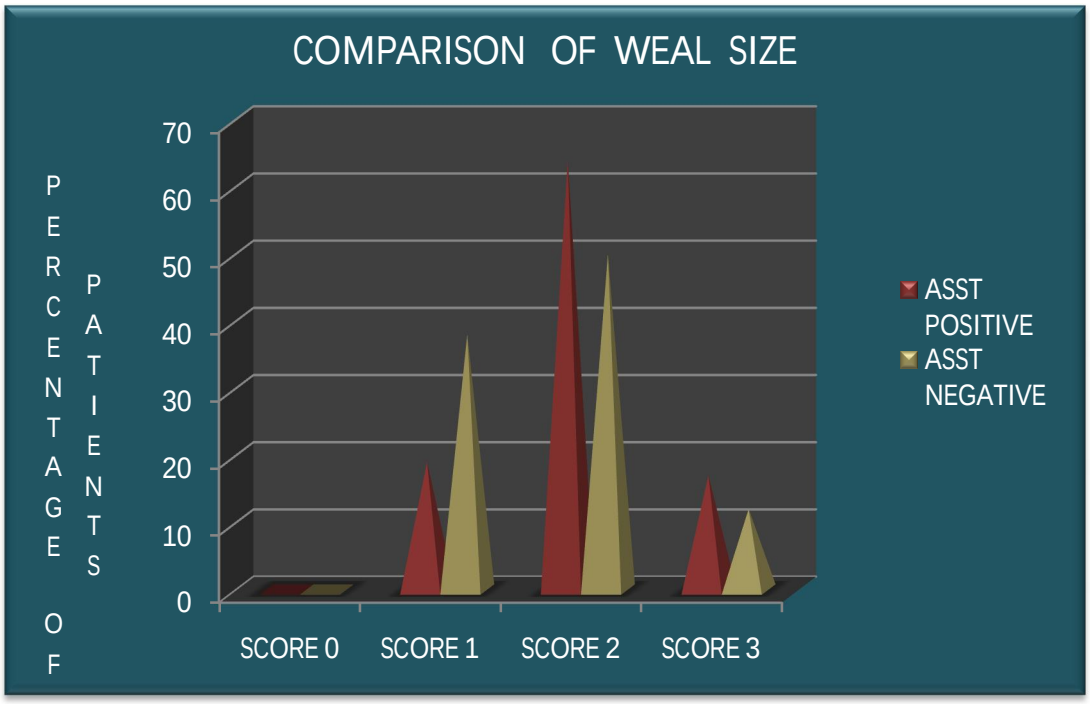
ASST RESULT	WEAL SIZE SCORE			P VALUE
	1	2	3	
Positive	9	30	8	0.057
Negative	58	76	19	
Total	67	106	27	

13. TOTAL SEVERITY SCORE (TSS)

TSS is calculated for a patient by adding all the six scores. In ASST positive patients 59.57 %(28 patients) had severe TSS score, 38.29 %(18 patients) had moderate TSS and 2.13 %(1 patient) had mild TSS. In ASST negative patients 68.62 %(105patients) had moderate TSS, 20.92 %(32 patients) had severe TSS and 10.46 %(16 patients) had mild TSS. There was significant difference in the TSS between groups, majority of ASST positive patients having severe TSS and majority of negative patients having moderate TSS score. The mean TSS between two groups was also significant.

Table 16 : Comparison of TSS grade between two groups

ASST RESULT	TSS GRADING (IN NUMBER OF PATIENTS)			P VALUE
	MILD	MODERATE	SEVERE	
Positive	1	18	28	< 0.001
Negative	16	105	32	
Total	17	123	60	


COMPARISON OF WEAL SIZE
PERCENTAGE OF PATIENTS
70
60
50
40
30
20
10
0
SCORE 0
SCORE 1
SCORE 2
SCORE 3
ASST POSITIVE
ASST NEGATIVE

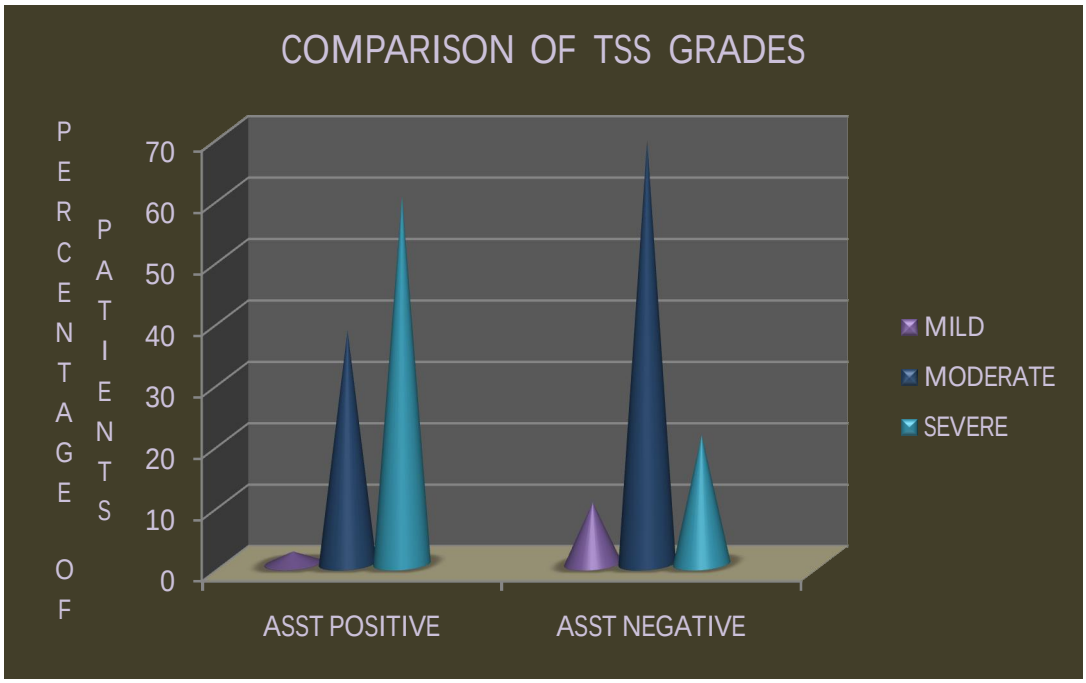

COMPARISON OF TSS GRADES
PERCENTAGE OF PATIENTS
70
60
50
40
30
20
10
0
ASST POSITIVE
ASST NEGATIVE
MILD
MODERATE
SEVERE

Table 17 : Comparison of mean TSS

ASST RESULT	NO OF PATIENTS	MEAN TSS	STD DEVIATION	P VALUE
Positive	47	13.829	2.884	<0.0001
Negative	153	10.79	2.925	

14. AUTOLOGOUS SERUM THERAPY (AST)

AST was offered to all ASST positive patients. One patient denied treatment. 46 patients were given AST. Only 44 completed 9 weeks of therapy, two patients dropped out. Of the 44 patients who completed treatment only 41 came for follow up, follow up lost for 3 patients.

Table 18 : Number of patients completed treatment and follow up visit

PARAMETERS	NO OF PATIENTS
Total asst positive	47
AST given	46
Completed 9 weeks of treatment	44
Follow up	41

At baseline majority of patients (25/57%) had severe symptoms and none of them was free from symptoms. Whereas At end of treatment none had severe TSS, 9(20%) were free from symptoms and majority had only mild TSS.

Table 19 : Comparison of mean and grade of TSS at different stages

TIME	NUMBER OF PATIENTS	TSS (MEAN)	TSS GRADE IN NO OF PATIENTS			
			CLEAR	MILD	MODERATE	SEVERE
Base line	44	13.68	0	1	18	25
End of treatment	44	3.68	9	31	4	0
Follow up	41	3.98	7	25	8	1

9 patients had complete clearance at the end of treatment. During follow up 7 out of 9 patients remained clear with no symptoms. Of the remaining 2 patients, follow up was lost for one patient and other patient had relapse of symptoms with TSS of 3. 1 patient relapsed back to have severe TSS. Though there was a slight increase in the mean TSS at follow up compared with the mean TSS at end of treatment, the difference was not statistically significant. None of the patients reached their baseline TSS value in the follow up period.

Table 20 : Comparison of mean TSS at base line and at end of treatment.

Time points	No of patients	Mean	Std. Deviation	P-Value
Base line	44	13.68	2.90	<0.0001
End of trt	44	3.68	2.55	

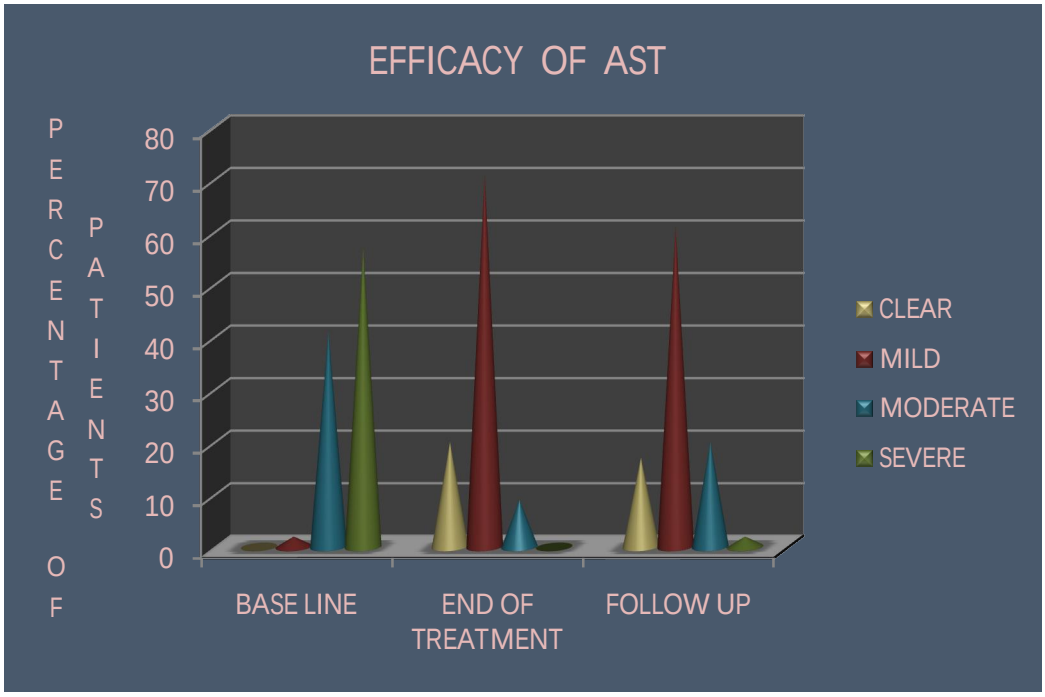
Table 21 : Comparison of mean TSS at end of treatment and at follow up.

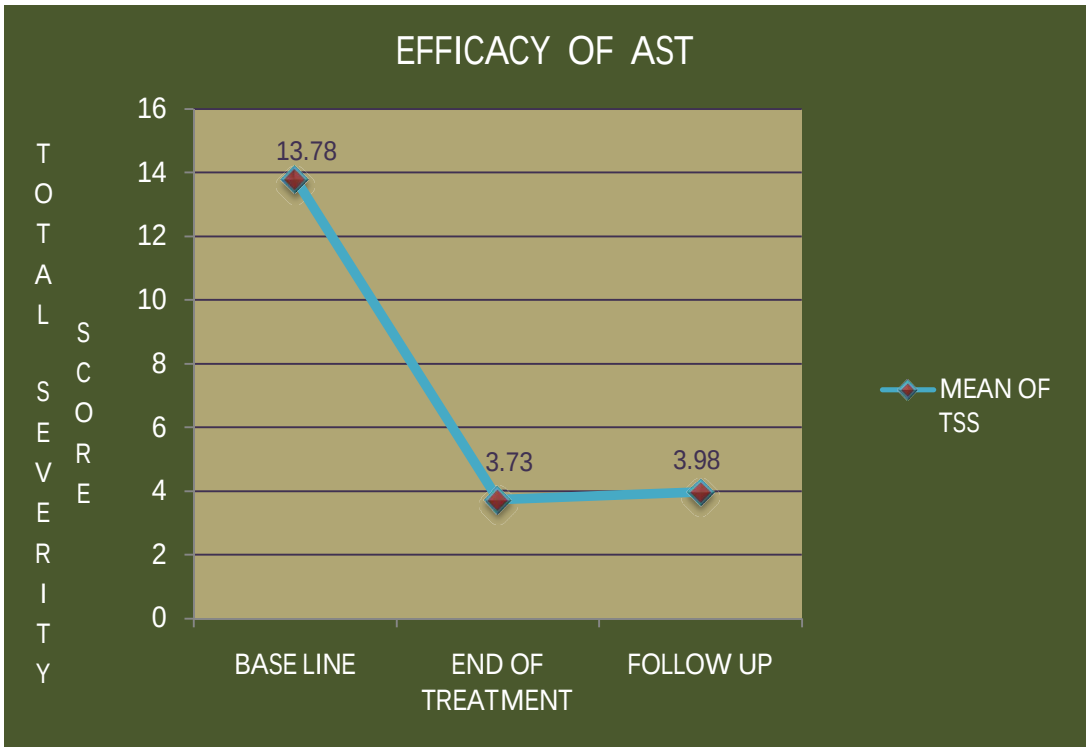
Time points	No of patients	Mean	Std. Deviation	P-Value
End of trt	41	3.73	2.55	0.681
Follow up	41	3.98	2.93	

Table 22 : Comparison of mean TSS at base line and at follow up.

Time points	No of patients	Mean	Std. Deviation	P-Value
Base line	41	13.78	2.97	<0.0001
Follow up	41	3.98	2.93	

The mean base line TSS value of 44 treatment completed patient was 13.83 and at end of treatment it was 3.68. Follow up mean TSS of 41 patients was 3.98. There was significant reduction in mean TSS on comparing base line with end of treatment (p value <0.0001) and base line with follow up (p value <0.0001). No significant difference was noted between mean TSS at end of treatment and follow up.


EFFICACY OF AST
PERCENTAGE OF PATIENTS
80
70
60
50
40
30
20
10
0
BASE LINE
END OF TREATMENT
FOLLOW UP
CLEAR
MILD
MODERATE
SEVERE


EFFICACY OF AST
TOTAL SEVERITY SCORE
16
14
12
10
8
6
4
2
0
13.78
3.73
3.98
BASE LINE
END OF TREATMENT
FOLLOW UP
MEAN OF TSS

Discussion

DISCUSSION

Chronic urticaria is a common disease, for which at times cause is not found. These patients with chronic idiopathic urticaria are sometimes difficult to treat. This study was done to find out patients with autoimmune etiology by doing ASST and to evaluate the efficacy of therapy with autologous serum in them.

Prevalence of autoimmune Urticaria

In our study with 200 chronic idiopathic urticaria patients, the prevalence of patients with autoimmune urticaria was 23.5 %.

Historically Grattan et al were the first to use ASST on 12 patients to differentiate chronic autoimmune urticaria from chronic idiopathic urticaria. Out of 12 patients 7 were positive in that study[9]. The reported prevalence of ASST positivity in patients of chronic urticaria ranges from 25-60% in various studies.

Study done by Bajaj et al in 394 patients showed ASST positivity in 49.5 %[5]. Godse et al conducted study on 45 patients and found 26.67% were ASST positive[116]. Out of 96 patients 53% were positive in a study by Asero et al[69].The prevalence of ASST positive patients in our study was little lower compared to other studies.

Sex distribution

In our study out of 47 ASST positive patients, 22 (47%) were males and 25(53%) were females. In ASST negative patients 58.82 % were females and 41.17% were males. Male to female ratio in ASST positive group was 0.8:1 and in ASST negative it was 1:1.42. There was no statistical significance in sex distribution between positive and negative group.

Increased female ratio in both groups noted in our study was comparable with the study conducted by Vohra et al in which female ratio was higher in both ASST positive [M: F; 1:2.07] and ASST negative [M: F; 1:1.25], but there was no statistical significance[117]. In other study by Zeinab abdel azim et al on 35 patients, all 15 ASST positive patients were females[118]. But no statistical significance was noted between positive and negative group.

Age distribution

Age of patients ranged from 12 to 71 years in our study. Mean age in ASST positive patients was 36.77±15 years and in ASST negative it was 35.42±13 years. There was no significant difference noted in our study which corroborates with the study conducted by zeinab et al in which age of patients ranged from 7 to 57 years[118]. Mean age noted in their study was

34±10 yrs for ASST positive patients and 30±11 years in ASST negative patients with no statistical significance. Our study was in concordance with studies by Bajaj et al and Zeinab et al[5,118].

Duration of disease

In our study duration of disease ranged from 2 – 180 months. Mean duration of disease in ASST positive group was 4.36±3 years and in ASST negative group was 3.58±3 years.

Mamatha et al and sabroe et al in their separate studies have noted decreased median duration of disease in ASST positive patients compared to negative ASST group[65]. However in a 5 year follow up study conducted by Toubi et al, ASST positive patients had longer duration of the disease[119]. Staubach et al also noted longer duration in ASST positive patients[89].

Though there was no statistical significance in the age distribution between two groups (P value = 0.1428) in our study, the mean duration of disease was longer in ASST positive patients which was comparable with studies by Toubi et al and Staubach et al[119,89].

Association with atopy

In our study 30 % of ASST positive patients and 28 % of ASST negative patients had atopy. There was no statistical significance.

In a study by Bajaj et al atopy was present in 46.7% of ASST positive patients and 38.5 % of ASST negative patients. No significant difference was noted[5]. In the Study by De Swerdt et al 32% of ASST positive patients and 31% of ASST negative patients had atopy. No significant difference was noted[120]. Our study results was in concordance with the results of the above studies.

Association with angiodema

In our study out of total 200 patients, 80 (40%) patients had angiodema. 28(59.57%) of ASST positive patients and 52(33.98%) of ASST negative had angioedema. The difference was statistically significant. In study by Vohra et al 59% of ASST positive patients and 52% of ASST negative patients had angioedema[117]. In a study by Zeinab et al 46.7 % of ASST positive and 40% of ASST negative patients had angioedema[118]. In both these studies the difference was insignificant. Whereas In a 5 years follow up study conducted by Toubi et al, 88% of angioedema positive patients and 80% of angiodema negative patients had urticaria at 12 month and at 5 years, 45% of patients with existing angiodema had urticaria compared to only 12% of angiodema negative patients. They noted a strong association between chronic urticaria duration and presence of angiodema[119].

Severity scores

Frequency score

In our study 68% of ASST positive patients had score 3 whereas only 21.56% of ASST negative patients had score 3. ASST positive patients had more frequent episodes of urticaria than negative group. Statistical significance was noted on comparing the frequency score between two groups ($P < 0.001$).

Our study results corroborates with the results of the study conducted by Mamatha et al, in which statistically significant difference in the frequency of attacks was noted on comparing ASST positive and ASST negative group with ASST positive patients having increased frequency($p=0.038$)[65]. Similar correlation with increased frequency and ASST positivity was noted in study by Sabroe et al[40].

Number of weals score

Out of 47 ASST positive patients, 3(6.34%) patients had score 1, 22(46.8 %) had score 2 and 22(46.8 %) had score 3. Out of 153 ASST negative patients 12(7.84%) had score 1, 73(41.71 %) had score 2 and 68(44.44%) had score 3. There was no statistical significance between two groups ($P=0.926$).

Our results correlated with the study by Bajaj et al in which there was no difference in the number of weals between ASST positive (mean=2.4±0.1) and ASST negative patients (mean=2.4±0.2)[5]. Whereas in a study by Sabroe et al significant difference in the number of weals score was noted with higher mean value in patients with histamine releasing activity (mean=2.3) compared to patients with no histamine releasing activity(mean=1.5)[40]. No significant difference in the mean was noted in our study between ASST positive (2.40 ± 0.6) and ASST negative (2.37 ± 0.6) groups.

Pruritus and AHT use

In the ASST positive group majority of patients had severe score for both pruritus and AHT use. In ASST negative group considerable number of patients had score 1(29 %) for pruritus and 13 patients (8.40%) had score 0 for AHT use. The difference was statistically significant with ASST positive patients having severe score for pruritus and higher requirement for antihistamines. There was also significant difference in the mean values for pruritus score (2.3 ± 0.6 for ASST positive and 1.98 ± 0.7 for ASST negative) and AHT use score (2.59 ± 0.6 for ASST positive and 1.42 ± 0.8 for ASST negative) in our study.

Our study results was in concordance with study by Staubach et al on chronic urticaria patients in which longer duration of disease and more antihistamine requirement was noted in ASST positive patients while size, number of weals and intensity was similar in ASST positive and ASST negative patients[89]. Sabroe et al in his study also noted significant difference in itching on a scale of 0-10, between histamine releasing (mean=9.5) and non histamine releasing (mean=8.0) patients[40]. Whereas our study results was not comparable with the study by Bajaj et al in which no significant difference was noted in pruritus and AHT use was noted between two groups[5].

Duration of weals

In our study in the ASST positive group around 28% of patients had score 3 and 32% had score 2. Percentage of patients having severe score was higher in ASST positive group while majority of ASST negative(54%) group had score 1. The difference was statistically significant (P=0.0015). Whereas no difference in the duration of weals was noted in studies by Bajaj et al and Staubach et al[5,89]. Duration of weals score was not included in the severity grading in studies by Toubi et al, Vohra et al and De Swerdt et al[119,117,120]. Further comparative clinical studies including duration of weals in the future are needed to compare the difference in the duration of weals between two groups.

Size of weals

In our study majority of ASST positive patients(64%) and ASST negative patients(50%) had score 2. There was no statistical significance between two groups in the weal size score (P=0.0623). Similar results with no difference in size of weals was noted in studies conducted by Bajaj et al and Staubach et al[5,89].

Total Severity Score

There was significant difference in the TSS between both groups in our study. Majority of ASST positive patients had severe TSS while majority of ASST negative patients had moderate TSS score. Percentage of patients having mild TSS was higher in ASST negative than ASST positive group.

Study by Bajaj et al with similar scoring system, as used in our study showed no significant difference in mean TSS between two groups[5]. But highly significant difference in the mean TSS was noted in our study with ASST positive patients having higher mean TSS indicating more severe disease in them. Different types of scoring system was used in various studies to calculate the severity of urticaria and to compare the difference in ASST positive and negative groups.

Sabroe et al in their study used number of weals, itching, systemic symptoms and weal distribution to calculate total severity score. Results obtained in their study was , patients with histamine releasing activity had more severe score than patients with no histamine releasing activity. The difference was statistically significant[40].

Study by Vohra et al also noted significantly higher urticaria activity score (UAS) in ASST positive patients than ASST negative patients. The study used pruritus score and weal score to calculate the UAS[117].

The difference in the significance/insignificance noted in difference studies may be due to the different parameters used and lack of uniformity. This denotes the need for standardization in the parameters used in urticaria for the calculation of total severity scores.

Autologous serum therapy

The mean base line TSS value of 44 treatment completed patient was 13.68 and at end of treatment it was 3.68. Follow up mean TSS of 41 patients was 3.98. There was significant reduction in mean TSS on comparing base line with end of treatment (p value <0.001) and base line with follow up (p value <0.0001).

Though there was increase in actual TSS score in some patients during follow up, it did not reach the base line value and the difference in the mean TSS value at end of treatment and follow up was insignificant.

Only few studies with autologous serum therapy on chronic urticaria patients are available. Staubach et al used autologous whole blood for therapy. Treatment was given using autologous whole blood weekly for eight weeks. They found significant reduction in severity scores in ASST positive patients (41%). ASST negative patients showed only 21% decrease in severity scores which was not different from placebo group[89].

Bajaj et al in his study used autologous serum for therapy instead of whole blood. The reason being, presence of autoreactive components in the serum but not in the cellular components of blood, serum can be injected using finer needles which increases compliance and immediate injection of whole blood before it clots requires increased patient co-operation. They noted AST was also effective in ASST negative patients. But ASST positive patients had dramatic decline in the severity score and lower TSS score at follow up compared to ASST negative group. AST was given weekly once for nine weeks and patients were followed for 12-16 weeks[5]. Our study had similar duration of therapy and follow up period, but mild increase in the mean TSS was noted in follow up. Whereas Bajaj et al noted further decline in mean TSS during follow up. But they also noted relapse in 2 of their patients during 12 and 18 months(more than their study follow up period). Variation in the relapse rate during follow up in our study compared with their study may be due to the smaller sample size in our study.

Since only limited studies are conducted on chronic urticaria patients using autologous serum/ autologous whole blood for therapy, large scale placebo controlled randomized studies with longer follow up period are needed to know the efficacy.

Limitations:

ASST positivity is only suggestive but not diagnostic of autoimmune urticaria. Due to the unavailability of basophil histamine release assay which is currently the gold standard test for detection of autoimmune urticaria, we used ASST which has a sensitivity of 70% and specificity of 80%. Since ASST positive patients in our study had severe disease, AST efficacy was evaluated without a placebo control which is the main limitation of our study.

Conclusion

CONCLUSION

1. Prevalence of autoimmune urticaria among patients with chronic idiopathic urticaria was 23.5%.

2. There was no difference in the epidemiological parameters like age and sex between ASST positive and ASST negative patients.

3. No difference in the duration of the disease was noted between ASST positive and ASST negative patients.

4. There was no significant difference in the association of atopy with ASST results.

5. Increased association with angiodema was noted in ASST positive patients compared to ASST negative patients.

6. ASST positive patients had more frequent episodes, severe pruritus, prolonged duration of weals, increased requirement for antihistamines and severe total severity score compared to ASST negative patients.

7. No significant difference was noted in the number of weals and size of weals between ASST positive and ASST negative patients.

8. AST was effective in complete clearance of disease in 20% patients. In the remaining, it was effective in considerably reducing the total severity of the disease.

9. 89 % of patients with complete clearance of the disease at the end of the treatment, remained clear during follow up also.

10. Mild increase in the mean severity score during follow up was noted compared to the mean severity score at the end of treatment. But the increase was statistically insignificant and none of the patients reached their initial base line score.

ASST is a simple, cost effective, easy to perform screening test which can be done as an outpatient procedure. It helps to identify autoimmune etiology, in patients previously categorized as having chronic idiopathic urticaria. It also provides evidence for the rationale use of immunomodulators in these patients.

In patients with chronic autoimmune urticaria, AST is a cheap, cost effective and potentially curative modality of treatment with no side effects. Especially in those patients with severe intensity who often require steroids and immunomodulators which are well known for their adverse effects on long term use, AST emerges to be a better therapeutic option.

Annexures

Bibliography

BIBLIOGRAPHY

1. Rook A, Warin RP, Champion RH. The historical background. In: Urticaria. London: *Saunders* 1974: 1-9.

2 . Goh CL, Tan KT. Chronic autoimmune urticaria : Where we stand ?. *Indian J Dermatol* 2009;54:269-74.

3. Ghosh SK, Ghosh S. Autologous serum skin test. *Indian J Dermatol* 2009;54:867.

4. Yadav S, Upadhyay A, Bajaj AK. Chronic urticaria: An overview. *Indian J Dermatol* 2006;51:171-7

5. Bajaj AK, Saraswat A, Upadhyay A, Damisetty R, Dhar S. Autologous serum therapy in chronic urticaria: Old wine in a new bottle. *Indian J Dermatol Venereol Leprol* 2008;74:109-13.

6. Hui SD, Fen WX, Wang N. Manual of Dermatology in Chinese Medicine. Eastland Press: Seattle, 1995; 204-213.

7. Cullen W. Synopsia Nosologiae Methodicae. Kincaid & Creech: Edinburgh, 1769.

8. Humphreys F. Major landmarks in the history of urticarial disorders. *Int J Dermatol* 1997; 36: 793-6.

9. Grattan CEH, Wallington TB, Warin RP, Kennedy CTC, Bradfield JW. A serological mediator in chronic idiopathic urticaria -- a clinical, immunological and histological evaluation. *Br J Dermatol* 1986; 114:583-590.

10. Hide M, Francis DM, Grattan CE, Hakimi J, Kochan JP, Greaves MW. Autoantibodies against the high affinity IgE receptor as a cause of histamine release in chronic urticaria. *N Engl J Med* 1993; 328:1599-604.

11. Schafer T, Ring I. Epidemiology of urticaria. In: Burr ML, editor. Epidemiology of clinical allergy: monographs in allergy. Basel: Karger, 1993: 49-60

12. Rook's Textbook of Dermatology, Eighth edition; vol 1, 22.2-22.25.

13. O"Donnell BF, O"Neill CM, Francis DM, et al. Human leucocyte antigen class 2 associations in chronic idiopathic urticaria. *Br J Dermatol* 1999; 140: 853-858.

14. Sabroe RA, Fiebiger E, Francis DM, et al Classification of anti-FcεR1 and anti-IgE autoantibodies in chronic idiopathic urticaria and correlation with disease severity. *J Allergy Clin Immunol* 2002; 110:492-499.

15. Grattan CEH, Francis DM, Barr RM, et al Plasmapheresis for severe unremitting chronic urticaria. *Lancet* 1992; 339:1078-1080.

16. The Role of Allergens and Pseudo allergens in Urticaria. *Journal of Investigative Dermatology Symposium Proceedings* 2001; 6: 132–134.

17. Juhlin L. Recurrent urticaria: clinical investigation of 330 patients. *Br J Dermatol* 1981; 104:369–381.

18. Grattan C. Aspirin sensitive Urticaria. *Clin Exp Dermatol* 2003;28:123-7

19. ZuberbierT, IfflanderJ, SemmlerC, et al acute Urticaria clinical aspects and therapeutic responsiveness. *Arch Dermatol* 1996; 76: 295 -29.

20. Aoki T, Kojima M, Horiko T et al,. Acute urticaria: History and natural course of 50 cases. *J Dermatol* 1994; 21: 73-77.

21. Ormerod AD, Reid TMS, Main RA. Penicillin in milk - its importance in urticaria. *Clin Allergy* 1987; 17: 229-234.

22. Barber HW et al,.Chronic urticaria and angioneurotic edema due to bacterial sensitization. *Br J Dermatol* 1923; 35:209-18.

23. Hizal M, Tuzun B, Wolf R, Tuzun Y**.** The relationship between Helicobacter pylori IgG antibody and autologous serum test in chronic urticaria. *Int J Dermatol* 2000; 39:443-5.

24. Gosh S, Kanwar AJ, Dhar S, et al. Role of gastrointestinal parasites in urticaria. *Indian J Dermatol Venereol Leprol* 1993; 59: 117-119.

25. Wolfrom E, Chêne G, Lejoly-Boisseau H, Beylot C, Geniaux M, Taïeb A. Chronic urticaria and toxocara canis infection. A case-control study. *Ann Dermatol Venereol* 1996; 123(4):240-6.

26. Numata T, Yanomota S, Yamura T. The role of mite allergen in chronic urticaria. *Ann Allergy* 1979; 43: 356-358.

27. Young E, Stoneham MD, Petruckevitch A, et al. A population study of food intolerance. *Lancet* 1994; 343: 1127-1130.

28. Clayton DE, Busse W. Anaphylaxis to wine. *Clin Allergy* 1980;10: 341-342.

29. Reisman RE. Insect stings. *N Engl J Med* 1994; 331: 523-527.

30. Kobza Black A, Champion RH. Urticaria. In: Textbook of Dermatology, Champion RH, Burton JL, Burns DA & Breathnac SM eds. Sixth edition, Blackwell Science, 1998; 2113-2139.

31. Stephens CJM, Black MM. Premenstrual eruptions: autoimmune progesterone dermatitis. *Semin dermatol* 1989; 8: 26-9.

32. Koblenzer C. Psychosomatic concepts in dermatology. A dermatologist-psychoanalyst's viewpoint. *Arch Dermatol* 1983; 119: 501-12.

33. Gupta MA, Gupta AK, Schork NJ, et al. Depression modulates pruritus perception: a study of pruritus in psoriasis, atopic dermatitis and chronic idiopathic urticaria. *Psychosom Med* 1994; 56: 3640.

34. Kuby. Textbook of immunology. Hypersensitivity reactions Chapter 15, Part 111. Pg 375 – 378.

35. Grattan C, Black AK. Urticaria and angioedema. In: Bolognia JL, Jorrizo JL, Rapini RP, editors. *Dermatology* 2003; Vol. 1: 287-302

36. Haas N, Schadendort D, Henz BM. Differential endothelial adhesion molecule expression in early and late whealing reactions. *Int Arch Allergy Immunol* 1998;115:210-4.

37. Lee KH, Kim JY, Kang DS, Choi YJ, Lee WJ, Ro JY. Increased expression of endothelial cell adhesion molecules due to mediator release from human foreskin mast cells stimulated by autoantibodies in chronic urticaria sera. *J Invest Dermatol* 2002;118:658-63.

38. Juhlin L, Michaelsson G. Cutaneous reaction to kallikrein, bradykinin and histamine in healthy subjects and in patients with Urticaria. *Acta Derm Venereol* 1969; 49:26-36.

39. Stinhoff M, Neisous U, Ikoma A, Fartasch M. Proteinase activated receptor 2 mediates itch: a novel pathway for pruritus in human skin. *J Neurosci* 2003; 23:6176-6180.

40. Sabroe RA, Poon E, Orchard GE et al. Cutaneous inflammatory cell infiltrate in chronic idiopathic urticaria: Comparison of patients with and without anti-FcϵRI or anti-IgE autoantibodies. *J Allergy Clin Immunol* 1999;103:484-493.

41. CEH Grattan. Basophils in Chronic Urticaria. *Journal of Investigative Dermatology Symposium Proceedings* 2001;6:139–140.

42. Zweimen et al. Characteristics of histamine-releasing activity in the sera of patients with chronic idiopathic Urticaria. *The Journal of Allergy and Clinical Immunology*. July 1996;Volume 98(1),Pages 89-98.

43. Kikuchi Y, Kaplan AP. A role for C5a in augmenting IgG-dependent histamine release from basophils in chronic urticaria. *J Allergy Clin Immunol* 2002; 109: 114-86.

44. Fiebiger E, Hammerschmid F, Stingl G, Maurer D. Anti-FcεRIα autoantibodies in autoimmune-mediated disorders: identification of a structure function relationship. *J Clin Invest* 1998;101:243-51.

45. Horn, M. P., Gerster, T., Ochensberger, B., Derer, T., Kricek, F., Jouvin, M. H., Kinet, J. P., Tschernig, T., Vogel, M., Stadler, B. M., et al. Human anti-FcεRIα auto-antibodies isolated from healthy donors cross react with tetanus toxoid. *Eur. J. Immunol* 1999;29:1139-1148.

46. O'Donnell, B. F., R. M. Barr, A. K. Black, D. M. Francis, F. Kermani, N. Niimi, R. J. Barlow, R. K. Winkelmann, M. W. Greaves.Intravenous immunoglobulin in autoimmune chronic urticaria. *Br. J. Dermatol* 1998;138*:*101.-106.

47. Michael p. HornJana m. Pachlopnik, Monique vogel, Markus dahinden, et al. Conditional autoimmunity mediated by human natural anti-FcεRIα autoantibodies?*The FASEB Journal* 2001;15:2268-2274.

48. Michihiro Hide, David M. Francis, Clive Grattan, John Hakimi, Jarema P. Kochan, and Malcolm W. Greaves. Autoantibodies against the High-Affinity IgE Receptor as a Cause of Histamine Release in Chronic Urticaria. *N Engl J Med* 1993; 328:1599-1604.

49. Soundararajan S, Kikuchi Y, Joseph K, et al. Functional assessment of pathogenic IgG subclasses in chronic autoimmune urticaria. *J Allergy Clin Immunol* 2005; 115: 815-21.

50. Ferrer M, Nakazawa K, Kaplan AP. Complement dependence of histamine release in chronic urticaria. *J Allergy Clin Immunol* 1999;104:169-72.

51. Füreder W, Agis H,Willheim M, Bankl HC, Maier U, Kishi K, et al. Differential expression of complement receptors on human basophils and mast cells. Evidence for mast cell heterogeneity and CD88/C5aR expression on skin mast cells. *J Immunol* 1995;155:3152-60.

52. chronic urticaria a review. *Am J Clin Dermatol* 2009;10(1): 9-21.

53. Kaplan AP. Chronic urticaria: pathogenesis and treatment. *J Allergy Clin Immunol* 2004; 114: 465-74

54. Peters MS, Schroeter AL, Kephart GM, Gleich GJ. Localization of eosinophil granule major basic protein in chronic urticaria. *J Invest Dermatol* 1983;81:39-43.

55. Chronic Urticaria. Malcolm Greaves, MD. *J allergy clin immunol* 2000 volume 105, number 4.

56. Champion RH. Acute and chronic urticaria. *Semin Dermatol* 1987; 6: 286 -291.

57. Sabroe RA, Seed PT, Francis DM, Barr RM, Black AK, Greaves MW. Chronic idiopathic urticaria: Comparison of the clinical features of patients with and without anti-FceRI or anti-IgE autoantibodies. *J Am Acad Dermatol* 1999;40:443-50.

58. Zauli D, Grassi A, Ballardini G, et al Thyroid autoimmunity in chronic idiopathic urticaria. *Am J Clin Dermatol* 2002; 3:525-528.

59. Y Levy, N Segal, N Weintrob, Y L Danon .Chronic urticaria: association with thyroid autoimmunity. *Arch Dis Child* 2003;88:517–519.

60. IADVL textbook of dermatology. Cutaneous vascular response, chapter 22, pg 657-673.

61. Surrinder Kaur, Srabani Ghosh, JA Kanwar, Incidence of Dental Caries in Chronic Urticaria. Gauba Year : 1991; Volume 57(5) Page : 276-278.

62. Pasricha JS, Pasricha A, Prakash O. Role of gastrointestinal parasites in urticaria. *Ann Allergy* 1972;30:348-51.

63. Chronic idiopathic Urticaria,Journal Article, Curr Opin Allergy ; *Clin Immunol* September 2003;52.

64. Vohra S, Sharma NL, Mahajan VK. Autologous serum skin test: Methodology, interpretation and clinical applications. *Indian J Dermatol Venereol Leprol* 2009;75:545-8.

65. Mamatha G, Balachandran C, Smitha P. Chronic idiopathic Urticaria: Comparison of clinical features with positive autologous serum skin test. *Indian J Dermatol Venereol Leprol* 2008;74: 105-8.

66. Ferrer M, Kinét JP, Kaplan AP. Comparative studies of functional and binding assays for IgG anti-FcεRIα (α-subunit) in chronic urticaria. *J Allergy Clin Immunol* 1998;101:672-6.

67. Umberto Fagiolo. Effects of complement inactivation and IgG depletion on skin reactivity to autologous serum in chronic idiopathic urticaria. *J allergy clin Immunol* september 2000;59.

68. Kikuchi Y, Kaplan AP. Mechanisms of autoimmune activation of basophils in chronic urticaria. *J Allergy Clin Immunol* 2001;107:1056-62.

69. Asero R, Tedeschi A, Riboldi P, Cugno P. Plasma of patients with chronic urticaria shows signs of thrombin generation, and its intradermal injection causes wheal-and-flare reactions much more frequently than autologous serum. *J Allergy Clin Immunol* 2006;117:1113-7.

70. Nimii N, Francis DM, Kermani F, et al Dermal mast cell activation by autoantibodies against the high affinity IgE receptor in chronic urticaria. *J Invest Dermatol* 1996; 106:1001-1010.

71. Tong LJ, Balakrishnan G, Kochan JP, et al Assessment of autoimmunity in patients with chronic urticaria. *J Allergy Clin Immunol* 1997; 99: 461-465.

72. Kessel A,Helou W et al. Elevated serum total IgE--a potential marker for severe chronic urticaria. *Int Arch Allergy Immunol* 2010; 153(3):288-93.

73. Kobza Black A, Champion RH. Urticaria. In: Textbook of Dermatology, Champion RH, Burton JL, Burns DA & Breathnac SM eds. Sixth edition, Blackwell Science, 1998; 2113-2139.

74. Godse KV, Zawar V, Krupashankar DS, Girdhar M, Kandhari S, Dhar S, Ghosh S, Rajagopalan M, Zuberbier T. Consensus statement on the management of urticaria. *Indian J Dermatol* 2011;56:485-9

75. Godse KV, Nadkarni NJ, Jani G, Ghate S. Fexofenadine in higher doses in chronic spontaneous urticaria; Indian *Dermatol Online J* 2010;1:45-6.

76. Godse KV. Updosing of antihistamines to improve control of chronic urticaria. *Indian J Dermatol Venereol Leprol* 2010;76:61-2.

77. Nathan RA, Mason J, Bernstein DI, Howland WC 3rd, Kaiser HB, Meltzer EO, et al. Long-Term Tolerability of Fexofenadine in Healthy Volunteers. *Clin Drug Invest* 1999;18;317-28.

78. Smith SM, Gums JG. Fexofenadine: Biochemical, pharmacokinetic and pharmacodynamic properties and its unique role in allergic disorders. *Expert Opin Drug Metab Toxicol* 2009;5:813-

79. Minocha YC, Minocha KB, Sood VK, Dogra A. Evaluation of H2 receptor antagonists in chronic idiopathic urticaria. *Indian J Dermatol Venereol Leprol* 1995;61:265-7.

80. NTIS. Use of chlorpheniramine in pregnancy. Newcastle upon type: National Teratology Information Service; Regional Drug and Therapeutics centre. 2002.

81. Dibbern DA Jr, Dreskin SC. Urticaria and angioedema: An overview. *Immunol Allergy Clin North Am* 2004;24:141-62.

82. Erbagci Z. The leucotriene receptor antagonist montelukast in the treatment of chronic idiopathic urticaria: A single blind, placebo controlled, crossover clinical study. *J Allergy Clin Immunol* 2002;110:484-8.

83. Ghosh S, Haldar S. Therapeutic effect of doxepin in chronic idiopathic urticaria. *Indian J Dermatol Venereol Leprol* 1990;56:218-20.

84. Greene SL, Reed CE, Schroeter AL. Double-blind crossover study comparing doxepin with diphenhydramine for the treatment of chronic urticaria. *J Am Acad Dermatol* 1985;12:669-75.

85. Tharp et al, chronic Urticaria: pathophysiology and treatment approaches. *J Allergy Clin Immunol* 1996; 98(6), part 3.

86. Paradis L, Lavoie A, Burnet C, et al. Effects of systemic corticosteroids on cutaneous histamine secretion and histamine releasing factor in patients with chronic idiopathic urticaria. *Clin Exp Allergy* 1996; 26: 815-820.

87. Inamadar AC, Palit A; Management of autoimmune Urticaria: *Indian J Dermatol Venereol Leprol* 2008:74:89-91.

88. Chopra A, Mamta, Chopra D. Autohaemotherapy in chronic urticaria. *Indian J Dermatol Venereol Leprol* 1995;61:323-4.

89. Staubach P, Onnen K, Vonend A, Metz M, Siebenhaar F, Tschentscher I, et al . Autologous whole blood injections to patients with chronic urticaria and a positive autologous serum skin test: A placebo-controlled trial. *Dermatology* 2006;212:150-9.

90. Bressler RB, Sowel K, Huston PP. Therapy of chronic idiopathic urticaria with nifedipine: demonstration of beneficial effect in a double-blind placebo controlled, cross-over trail. *J Allergy Clin Immunol* 1989; 83: 756-63.

91. Fradin MS, Ellis CN, Goldfarb MT et al. Oral Cyclosporine for severe chronic idiopathic urticaria and angioedema. *J Am Dermatol* 1991; 125: 1065-67.

92. Toubi E, Blant A, Kessel A et al. Low dose cyclosporine in the treatment of chronic idiopathic urticaria. *Allergy* 1997; 52: 312-16.

93. Grattan CEH, O'Donnell BF, Francis DM et al. Randomized double blind study of cyclosporine in chronic idiopathic urticaria. *Br J Dermatol* 2000; 143: 365-72.

94. Cyclosporine in chronic idiopathic urticaria: a double-blind, randomized, placebo-controlled trial. Vena GA, et al. *J Am Acad Dermatol* 2006; 55:705-709.

95. Di Giacchino M, Di Stefano F, Cavallucci E, Verna N, Ramondo S, Paolini F, et al. Treatment of chronic idiopathic urticaria and positive autologous serum skin test with cyclosporine: Clinical and immunological evaluation. *Allergy Asthma Proc* 2003; 24:285-90.

96. Gach JE, Sabroe RA, Greaves MW, Black AK. Methotrexate responsive chronic idiopathic urticaria: a report of two cases. *Br J Dermatol* 2001; 145: 340-43.

97. Kiran Godse, Methotrexate in autoimmune Urticaria. *Indian journal dermatol venerol leprol* 2004, vol 70(6).

98. Parslew R, Pryce D, Ashworth J, Friedmann PS. Warfarin treatment of chronic idiopathic urticaria and angio-oedema. *Clin Exp Allergy* 2000; 30:1161-5. 11.

99. Warfarin sodium therapy for chronic urticaria and angioedema. *South Med J* 1986; 79:389.

100. Asero R, Tedeschi A, Coppola R, Griffini S, Paparella P, Riboldi P, et al. Activation of the tissue factor pathway of blood coagulation in patients with chronic urticaria. *J Allergy Clin Immunol* 2007; 119:705-10.

101. Mahesh PA, Pudupakkam VK, Holla AD, Dande T. Effect of warfarin on chronic idiopathic urticaria. *Indian J Dermatol Venereol Leprol* 2009; 75:187-9Duvall LA, Boackle RJ, King RG.

102. Godse KV. Omalizumab in severe chronic urticaria. *Indian J Dermatol Venereol* 2008; 74:157-8.

103. Kaplan et al, Treatment of chronic autoimmune urticaria with Omalizumab. *J allergy clin Immunol* september 2008, vol 122(3), pg 563-573.

104. Spector SL, Tan RA. Effect of omalizumab on patients with chronic urticaria. *Ann Allergy Asthma Immunol* 2007; 99:190-3.

105. Klote MM, Nelson MR, Engler RJ. Autoimmune urticaria response to high dose intravenous immunoglobulin. *Ann Allergy Asthma Immunol* 2005; 94:307-8.

106. Rütter A, Luger TA. High-dose intravenous immunoglobulins: An approach to treat severe immune-mediated and autoimmune diseases of the skin. *J Am Acad Dermatol* 2001; 44:1010-24.

107. Bernstein JA, Garramone SM, Lower EG. Successful treatment of autoimmune chronic idiopathic uticaria with intravenous cyclophosphamide. *Ann Allergy Asthma Immunology* 2002; 89: 212-14.

108. Boehm I, Bauer R, Bieber T. Urticaria treated with dapsone. *Allergy* 1999;54:765-6.

109. Thorman J, Laurberg, Zachariae H. Oral sodium chromoglycate in chronic urticaria. *Allergy* 1980; 54: 669-74.

110. Rumbyri IS, Katz JL, Schoket AL. Resolution of chronic urticaria in patients with thyroid autoimmunity. *J Allergy Clin Immunol* 1996; 96: 901-905.

111. Reeves GE, Boyle MJ, Bonfield J, Dobson P, Loewenthal M. Impact of hydroxychloroquine therapy on chronic urticaria: Chronic autoimmune urticaria study and evaluation. *Intern Med J* 2004;34:182-6.

112. Kessel A, Bamberger E, Toubi E. Tacrolimus in the treatment of severe chronic idiopathic urticaria an open-label prospective study. *J Am Acad Dermatol* 2005;52:145-8.

113. Shahar E, Bergman R, Guttman-Yassky E, Pollack S. Treatment of severe chronic idiopathic urticaria with oral mycophenolate mofetil in patients not responding to antihistamines and/or corticosteroids. *Int J Dermatol* 2006;45:1224-7.

114. Olafsson JH, LarkoÈ O, Roupe G, Granerus G, Bengtsson U. Treatment of chronic urticaria with angio-oedema with PUVA or UVA plus placebo: a double-blind study. *Arch Dermatol Res* 1986;278: 228-31.

115. Hartmann K, Hani N, Hinrichs R, Hunzelmann N, Scharffetter-Kochanek K. Successful sulfasalazine treatment of severe chronic idiopathic urticaria associated with pressure urticaria. *Acta Dermatol Venereol* 2001;81:71.

116. Godse KV. Autologous serum skin test in chronic idiopathic urticaria. *Indian J Dermatol Venereol Leprol* 2004;70:283-4.

117. Vohra S, Sharma NL, Mahajan VK, Shanker V. Clinicoepidemiologic features of chronic urticaria in patients having positive versus negative autologous serum skin test: A study of 100 Indian patients. *Indian J Dermatol Venereol Leprol* 2011;77:156-9.

118. Zeinab Abdel Azim M.D.,Shaymaa El Mongy M.D. and Hanan Salem M.D. Autologous Serum Skin Test in Chronic Idiopathic Urticaria: Comparative Study in Patients with Positive versus Negative Test. *J Egypt Women Dermatol Soc* 2010 Vol. 7(2).

119. E. Toubi, A. Kessel, N. Avshovich,E. Bamberger, E. Sabo, D. Nusem,J. Panasoff. Clinical and laboratory parameters in predicting chronic urticaria duration: a prospective study of 139 patients. *Allergy* 2004: 59: 869–873.

120. Ann De Swerdt, MD, et al, Detection of basophil-activating IgG autoantibodies in chronic idiopathic urticaria by induction of CD63. *J Allergy Clin Immunol* volume 116, number 3.

Proforma

PROFORMA

Name: Age: Sex: Occupation:

O.P No:

Address: Phone No:

PRESENTING COMPLAINTS:

H/O PRESENTING ILLNESS:

H/O total duration of illness:

H/O itching:

H/O frequency of episodes:

H/O number of weals:

H/O pain:

H/O duration of weals:

H/O frequency of AHT use:

H/O size of weal:

H/S/O atopy:

H/O angiodema:

H/S/O respiratory tract infections:
(Sinusitis/headache/sore throat)

H/O any aggravating foods:

H/S/O gastritis:

H/O drug intake:

H/O allergy to house dust/pollen/animal danders:

H/O episodes following exposure to cold/heat/pressure/sunlight:

H/O premenstrual exacerbation:

H/O weals following exercise:

H/O thyroid problem:

H/O dental/ortho implants:

H/O breathing difficulties/difficulty in swallowing during episodes:

H/S/O any recent illness:

PAST HISTORY:

H/O diabetes/hypertension/tuberculosis/bronchial asthma.

PERSONAL HISTORY:

Diet:

Alcoholism:

Smoking:

FAMILY HISTORY:

H/O atopy

H/O autoimmune diseases:

MENSTRUAL HISTORY:

TREATMENT HISTORY:

GENERAL EXAMINATION:

Built:

Nourishment:

Anemia/jaundice/clubbing/pedal edema/dyspnoea/lymphadenopathy

BP: Pulse: Resp rate:

CVS:

Respiratory system:

Abdominal examination:

CNS:

Endocrine system:

DERMATOLOGICAL EXAMINATION:

Description and extent of lesions:

Palms and soles:

Mucosa:

Hair:

Nails:

OPINION:

ENT

Dental to rule out focal sepsis

INVESTIGATIONS:

Complete blood count

Hb:

Total count:

Differential count:

ESR:

Urine routine:

Liver function test:

Renal function test:

Stool examination:

Thyroid function tests (if required):

USG abdomen (if required):

Master chart

MASTER CHART

S. no	AGE	SEX	DURATION	FREQUENCY SCORE	NO OF WEAL SCORE	PRURITUS SCORE	WEAL DURATION	AHT USE SCORE	WEAL SIZE SCORE	ATOPY	ANGIO ODEMA	TSS	ASST RESULT	BASE LINE	END OF TREATMENT	FOLLOW UP
1	20	F	6 Mn	2	3	1	2	2	2	P	P	12	N	-	-	-
2	50	F	10 Y	3	3	3	2	3	2	A	P	16	P	16	4	2
3	71	M	3 Mn	1	1	3	2	2	3	A	A	12	N	-	-	-
4	52	M	8 Mn	3	2	3	2	3	3	A	A	16	P	16	0	0
5	20	F	6 Mn	1	2	2	3	1	3	P	A	12	N	-	-	-
6	53	M	3 Y	3	2	2	3	3	3	P	P	16	N	-	-	-
7	18	M	6 Mn	1	1	2	2	1	3	A	A	10	N	-	-	-
8	34	F	1 Y	2	2	2	1	2	2	A	A	11	N	-	-	-
9	30	F	2 Y	3	2	2	1	3	1	A	P	12	N	-	-	-
10	23	F	2 Mn	1	3	1	1	1	2	P	A	9	N	-	-	-
11	19	F	3 Mn	1	3	1	1	1	2	A	P	9	N	-	-	-
12	32	M	2 Mn	2	1	1	1	2	1	P	P	8	P	8	3	3
13	22	M	2 Mn	2	2	3	2	2	1	A	P	12	N	-	-	-
14	30	F	3 Y	2	3	2	2	2	1	A	A	12	N	-	-	-
15	33	F	3 Mn	1	3	1	1	1	2	A	A	9	N	-	-	-
16	45	F	3 Mn	2	2	2	2	1	2	P	P	11	N	-	-	-
17	37	F	2 Y	3	2	1	2	3	3	A	A	14	N	-	-	-
18	35	M	13 Y	3	3	3	3	3	2	A	A	17	P	17	6	6
19	36	F	3 Mn	2	2	2	1	3	2	A	P	12	P	12	3	3
20	40	M	3 Y	1	2	2	2	1	2	A	A	10	N	-	-	-
21	21	F	3 Y	1	3	1	1	1	2	P	P	9	N	-	-	-
22	55	F	6 Mn	1	3	1	1	1	2	A	A	9	N	-	-	-
23	68	M	5 Y	3	3	2	2	3	2	A	P	15	P	15	3	3
24	38	F	7 Y	1	3	2	2	1	2	P	A	11	N	-	-	-
25	24	F	1Y	1	3	1	1	1	2	A	P	9	N	-	-	-
26	30	F	4 Y	1	2	2	1	1	2	A	P	9	N	-	-	-
27	27	M	3 Mn	1	3	1	1	1	2	A	A	9	N	-	-	-
28	19	M	10 Y	3	3	3	3	3	2	P	P	17	P	17	5	7

S. no	AGE	SEX	DURATION	FREQUENCY SCORE	NO OF WEAL SCORE	PRURITUS SCORE	WEAL DURATION	AHT USE SCORE	WEAL SIZE SCORE	ATOPY	ANGIO ODEMA	TSS	ASST RESULT	BASE LINE	END OF TREATMENT	FOLLOW UP
29	35	F	2 Y	1	2	2	2	1	2	A	A	10	N	-	-	-
30	50	F	6 Mn	3	3	3	3	3	2	A	P	17	N	-	-	-
31	29	M	15 Y	1	2	1	1	1	2	A	A	8	N	-	-	-
32	31	F	4 Y	2	3	2	2	2	1	P	A	12	N	-	-	-
33	29	F	2 Y	1	2	1	1	1	1	A	P	7	N	-	-	-
34	70	M	6 Mn	2	2	3	1	2	2	P	A	12	P	12	6	4
35	31	M	2 Y	3	2	3	3	3	3	A	P	17	P	17	2	3
36	37	M	10 Y	1	2	2	2	1	2	P	P	10	N	-	-	-
37	48	F	10 Y	1	2	1	1	1	3	A	A	9	N	-	-	-
38	34	F	2 Mn	3	1	2	2	2	2	A	P	12	N	-	-	-
39	15	F	5 Mn	3	3	3	1	2	3	A	A	15	P	15	-	-
40	35	F	3 Mn	1	2	2	1	1	2	A	A	9	N	-	-	-
41	21	M	1 Y	2	2	2	1	1	2	P	A	10	P	10	0	3
42	50	F	3 Mn	2	3	2	3	1	1	A	A	12	N	-	-	-
43	28	M	6 Mn	1	1	1	1	0	1	A	A	5	N	-	-	-
44	38	F	4 Y	3	2	3	2	3	2	A	P	15	P	15	2	3
45	24	M	1.5 Y	1	3	2	1	1	1	A	A	9	N	-	-	-
46	20	M	3 Y	2	3	2	2	2	3	A	A	14	N	-	-	-
47	54	F	5 Mn	3	2	2	1	3	1	A	P	12	P	12	3	2
48	30	M	4 Y	3	3	3	2	3	3	P	P	17	P	17	3	4
49	27	M	4 Mn	1	3	1	1	1	1	P	P	8	N	-	-	-
50	27	F	1.5 Y	2	2	2	2	1	1	A	A	10	N	-	-	-
51	40	F	8 Mn	3	3	3	2	2	3	A	A	16	N	-	-	-
52	55	F	2 Mn	1	2	1	1	0	1	A	A	6	N	-	-	-
53	24	F	3 Mn	3	3	2	2	2	3	P	P	15	N	-	-	-
54	27	M	4 Mn	2	2	2	3	1	2	P	P	12	P	12	6	6
55	19	M	8 Mn	1	1	1	1	0	1	P	A	5	N	-	-	-
56	30	M	1 Y	3	2	2	1	2	2	A	P	12	N	-	-	-
57	36	F	4 Mn	1	3	1	1	1	2	A	A	9	N	-	-	-
58	50	F	1 Y	3	2	2	3	3	2	P	A	15	P	15	0	0

S. no	AGE	SEX	DURATION	FREQUENCY SCORE	NO OF WEAL SCORE	PRURITUS SCORE	WEAL DURATION	AHT USE SCORE	WEAL SIZE SCORE	ATOPY	ANGIO ODEMA	TSS	ASST RESULT	BASE LINE	END OF TREATMENT	FOLLOW UP
59	33	M	3 Mn	1	1	1	1	0	1	A	A	5	N	-	-	-
60	57	F	1 Y	2	3	2	3	1	1	P	A	12	N	-	-	-
61	32	M	1.5 Mn	1	1	2	2	1	2	A	P	9	N	-	-	-
62	33	F	5 Mn	3	2	3	2	3	3	A	A	16	N	-	-	-
63	19	M	2 Y	3	3	3	3	3	3	P	P	18	N	-	-	-
64	20	M	1 Y	2	2	2	2	2	2	A	P	12	P	12	6	6
65	67	F	1 Y	2	2	1	3	1	1	A	A	10	N	-	-	-
66	66	F	3 Y	1	2	1	2	1	1	A	P	8	N	-	-	-
67	63	M	5 Mn	1	3	1	1	1	2	A	A	9	N	-	-	-
68	38	F	6 Mn	1	2	1	1	0	1	A	A	6	N	-	-	-
69	22	F	3 Y	3	3	3	3	3	2	P	P	17	N	-	-	-
70	65	F	3 Mn	1	3	1	1	1	1	A	A	8	N	-	-	-
71	53	M	3 Y	2	3	2	2	3	2	P	A	14	N	-	-	-
72	65	F	3 Mn	2	3	3	2	1	1	A	A	12	N	-	-	-
73	36	F	1 Y	3	2	2	2	3	2	A	P	14	N	-	-	-
74	35	F	5 Mn	3	3	3	3	3	3	A	P	18	P	18	6	7
75	35	F	2 Mn	1	3	1	1	1	1	P	P	8	N	-	-	-
76	55	F	2 Mn	1	1	1	1	0	1	A	A	5	N	-	-	-
77	33	M	1.5 Y	2	2	2	2	2	3	P	P	13	N	-	-	-
78	40	M	6 Mn	2	2	3	2	2	3	A	A	14	N	-	-	-
79	22	F	6 Mn	2	3	2	2	2	3	A	A	14	N	-	-	-
80	45	F	1.5 Y	3	3	2	2	3	1	A	A	14	P	14	3	6
81	23	M	3 Mn	1	3	1	1	1	1	A	P	8	N	-	-	-
82	28	M	2 Y	1	3	1	1	1	1	A	A	8	N	-	-	-
83	18	M	6 Mn	3	2	2	3	3	2	P	P	15	N	-	-	-
84	26	F	3 Y	3	3	2	2	3	2	A	A	15	N	-	-	-
85	40	M	2 Mn	1	3	1	1	1	1	P	A	8	N	-	-	-
86	59	M	3 Mn	3	3	2	1	3	3	A	P	15	P	15	3	3
87	27	F	2 Mn	3	2	2	1	3	1	P	A	12	P	12	3	3
88	36	M	6 Y	1	2	2	1	1	2	P	A	9	N	-	-	-

S. no	AGE	SEX	DURATION	FREQUENCY SCORE	NO OF WEAL SCORE	PRURITUS SCORE	WEAL DURATION	AHT USE SCORE	WEAL SIZE SCORE	ATOPY	ANGIO ODEMA	TSS	ASST RESULT	BASE LINE	END OF TREATMENT	FOLLOW UP
89	16	M	3 Mn	1	3	3	1	1	1	A	A	10	N	-	-	-
90	40	F	3 Mn	3	2	2	1	3	1	P	A	12	N	-	-	-
91	24	M	2 Mn	1	3	3	1	1	1	A	P	10	N	-	-	-
92	28	M	3 Mn	1	3	1	1	1	1	A	A	8	N	-	-	-
93	41	F	2 Y	2	2	1	3	2	2	P	P	12	N	-	-	-
94	67	M	12 Y	3	2	2	2	2	2	A	P	13	N	-	-	-
95	18	M	3 Y	1	1	1	1	1	1	A	A	6	N	-	-	-
96	30	M	10 Y	2	3	2	1	1	1	P	P	10	N	-	-	-
97	50	F	3 Mn	1	1	1	1	1	1	A	A	6	N	-	-	-
98	40	F	6 Mn	3	3	3	2	3	2	P	P	16	P	16	6	8
99	50	F	6 Mn	3	2	2	1	2	1	A	A	11	N	-	-	-
100	32	F	3 Y	2	3	2	2	1	2	P	A	12	N	-	-	-
101	56	F	2 Y	1	2	1	1	1	2	A	A	8	N	-	-	-
102	45	M	1 Y	1	3	3	1	1	1	A	P	10	N	-	-	-
103	18	F	1 Y	3	2	1	1	3	1	A	A	11	P	11	3	4
104	40	F	7 Mn	3	2	1	1	3	2	A	A	12	N	-	-	-
105	21	M	1 Y	3	3	2	2	3	2	A	P	15	P	15	-	-
106	26	F	1 Y	3	2	2	1	2	2	A	A	12	N	-	-	-
107	45	F	1 Y	1	1	1	1	0	1	A	A	5	N	-	-	-
108	50	F	2 Mn	1	3	1	1	1	2	P	P	9	N	-	-	-
109	23	F	4 Mn	2	3	2	2	1	1	A	A	11	N	-	-	-
110	13	F	4 Mn	2	3	2	2	2	1	A	A	12	N	-	-	-
111	30	F	2 Mn	3	3	2	2	2	2	P	P	14	N	-	-	-
112	45	F	2 Y	2	2	3	1	1	2	P	A	11	N	-	-	-
113	40	F	8 Mn	3	2	2	1	2	2	A	P	12	N	-	-	-
114	18	F	1 Y	2	2	2	1	2	1	P	A	10	P	10	5	3
115	29	M	3 Y	2	2	3	2	1	2	A	A	12	N	-	-	-
116	25	M	2 Mn	1	2	1	1	0	1	A	A	6	N	-	-	-
117	42	F	10 Y	1	3	3	1	1	1	P	P	10	N	-	-	-
118	32	M	3 Mn	2	3	3	2	1	1	A	A	12	N	-	-	-

S. no	AGE	SEX	DURATION	FREQUENCY SCORE	NO OF WEAL SCORE	PRURITUS SCORE	WEAL DURATION	AHT USE SCORE	WEAL SIZE SCORE	ATOPY	ANGIO ODEMA	TSS	ASST RESULT	BASE LINE	END OF TREATMENT	FOLLOW UP
119	28	F	5 Mn	2	2	3	2	1	2	A	A	12	N	-	-	-
120	43	F	1 Y	1	3	3	1	1	1	P	P	10	N	-	-	-
121	50	M	10 Y	3	3	3	3	3	2	A	P	17	P	17	0	0
122	23	M	2 Y	3	3	3	2	3	2	A	P	16	P	16	0	0
123	54	F	4 Y	3	2	2	3	3	2	A	P	15	P	15	6	6
124	38	F	3 Y	2	2	2	2	2	2	P	P	12	P	12	4	0
125	26	F	5 Y	2	3	2	1	2	2	A	P	12	P	12	3	3
126	43	F	10 Y	3	3	3	3	3	2	A	P	17	P	17	8	8
127	52	F	8 Y	3	3	2	2	3	2	A	A	15	P	15	10	13
128	25	F	3 Mn	2	2	2	2	2	2	P	A	12	N	-	-	-
129	25	M	8 Mn	3	2	2	1	2	2	P	P	12	N	-	-	-
130	30	M	1 Y	3	2	3	2	3	2	A	A	15	N	-	-	-
131	19	F	5 Mn	1	2	1	1	0	1	A	A	6	N	-	-	-
132	24	M	9 Mn	1	3	3	1	1	3	P	A	12	N	-	-	-
133	34	M	2 Y	2	3	2	2	1	2	P	P	12	N	-	-	-
134	33	M	3 Mn	3	2	1	1	3	1	A	A	11	P	11	3	3
135	31	F	4 Mn	2	3	2	1	1	1	A	A	10	N	-	-	-
136	47	M	3 Mn	2	2	2	1	2	2	P	P	11	P	11	6	8
137	55	F	4 Mn	1	1	1	1	1	1	A	P	6	P	6	0	0
138	30	M	2 Y	3	2	2	2	2	2	A	A	13	N	-	-	-
139	40	M	3 Y	2	2	2	1	1	2	A	A	10	N	-	-	-
140	37	F	6 Mn	2	2	2	1	2	2	A	P	11	N	-	-	-
141	40	F	2 Mn	1	3	3	1	1	1	A	A	10	N	-	-	-
142	30	F	3 Mn	2	2	3	1	2	2	A	A	12	N	-	-	-
143	16	F	4 Mn	3	2	2	1	3	2	A	A	13	N	-	-	-
144	27	F	3 Y	2	2	2	2	2	2	A	A	12	N	-	-	-
145	29	F	7 Mn	2	1	1	1	2	1	A	A	8	P	8	3	3
146	55	M	3 Y	3	3	3	2	3	2	A	A	16	N	-	-	-
147	40	F	3 Mn	2	2	3	1	1	2	P	P	11	N	-	-	-
148	42	M	1 Y	2	2	2	2	2	2	P	A	12	N	-	-	-

S. no	AGE	SEX	DURATION	FREQUENCY SCORE	NO OF WEAL SCORE	PRURITUS SCORE	WEAL DURATION	AHT USE SCORE	WEAL SIZE SCORE	ATOPY	ANGIO ODEMA	TSS	ASST RESULT	BASE LINE	END OF TREATMENT	FOLLOW UP
149	50	F	2 Mn	1	2	1	1	0	1	A	P	6	N	-	-	-
150	30	M	6 Mn	2	2	3	2	1	2	A	A	12	N	-	-	-
151	43	F	4 Mn	2	2	2	2	2	1	P	P	11	N	-	-	-
152	50	M	2 Mn	2	3	2	1	1	2	A	A	11	N	-	-	-
153	30	F	4 Y	1	1	1	1	1	1	A	A	6	N	-	-	-
154	45	F	1 Y	1	2	1	1	0	1	A	A	6	N	-	-	-
155	39	F	2 Mn	3	2	3	2	2	2	A	A	14	N	-	-	-
156	26	M	2 Y	3	2	2	1	3	2	A	A	13	P	13	3	-
157	17	M	2 Y	2	2	2	2	2	2	A	P	12	P	12	0	-
158	60	M	3 Y	3	2	2	1	3	2	A	A	13	P	13	7	6
159	35	F	1 Y	3	3	3	3	2	2	A	A	16	P	16	8	8
160	32	F	6 Mn	3	3	3	3	3	3	A	P	18	P	18	-	-
161	30	M	4 Y	2	2	3	1	2	2	A	P	12	P	12	6	-
162	40	F	5 Y	3	3	3	2	3	2	A	P	16	P	16	0	0
163	29	M	2 Y	2	2	2	2	2	2	P	A	12	P	12	4	6
164	18	F	10 Mn	3	3	2	1	3	2	A	P	14	P	14	4	4
165	24	M	3 Y	3	3	3	3	3	2	A	A	17	P	17	0	0
166	56	F	10 Y	3	3	3	3	3	3	P	P	18	P	18	6	6
167	32	F	1 Y	2	3	2	2	2	3	A	A	14	N	-	-	-
168	40	M	2 Y	2	2	3	1	2	2	A	A	12	N	-	-	-
169	28	M	11 Mn	2	2	2	1	1	2	A	A	10	N	-	-	-
170	40	M	2 Y	3	3	3	3	2	3	A	A	17	N	-	-	-
171	28	F	3 Mn	1	3	3	1	1	1	P	P	10	N	-	-	-
172	28	M	1 Y	2	2	2	1	1	2	A	A	10	N	-	-	-
173	23	F	3 Mn	1	3	3	1	1	1	A	A	10	N	-	-	-
174	40	F	8 Mn	2	3	2	2	1	2	A	P	12	N	-	-	-
175	16	F	1 Y	3	3	2	2	2	2	A	A	14	N	-	-	-
176	55	F	2 Y	3	3	3	3	2	2	P	P	16	N	-	-	-
177	38	M	5 Y	2	2	3	1	2	2	A	A	12	N	-	-	-
178	27	M	2 Mn	2	2	3	2	2	2	A	A	13	N	-	-	-

S. no	AGE	SEX	DURATION	FREQUENCY SCORE	NO OF WEAL SCORE	PRURITUS SCORE	WEAL DURATION	AHT USE SCORE	WEAL SIZE SCORE	ATOPY	ANGIO ODEMA	TSS	ASST RESULT	BASE LINE	END OF TREATMENT	FOLLOW UP
179	29	F	8 Mn	2	2	2	1	2	1	A	A	10	N	-	-	-
180	32	M	3 Mn	1	3	3	1	1	1	A	A	10	N	-	-	-
181	35	F	6 Mn	1	3	3	1	1	1	P	P	10	N	-	-	-
182	24	F	1 Y	2	2	2	1	1	3	A	A	11	N	-	-	-
183	19	F	1 Y	2	3	3	2	2	2	A	P	14	N	-	-	-
184	51	M	2 Mn	1	3	3	1	1	1	A	A	10	N	-	-	-
185	28	M	6 Mn	2	2	1	2	1	2	A	A	10	N	-	-	-
186	46	F	1.5 Y	2	2	1	1	1	2	A	P	9	N	-	-	-
187	15	M	2 Y	2	2	3	1	2	1	A	P	11	N	-	-	-
188	40	M	3 Y	1	3	3	1	1	1	A	A	10	N	-	-	-
189	46	F	4 Mn	3	2	2	2	2	2	A	A	13	N	-	-	-
190	32	F	7 Mn	1	2	3	2	1	3	P	A	12	N	-	-	-
191	38	M	6 Y	*2*	2	2	2	1	2	A	P	11	N	-	-	-
192	51	M	10 Y	3	3	3	2	3	2	A	A	16	N	-	-	-
193	49	F	1 Y	3	3	2	2	2	2	A	A	14	N	-	-	-
194	21	F	3 Mn	2	2	2	2	1	2	A	A	11	N	-	-	-
195	26	F	2 Mn	1	3	3	1	1	1	A	P	10	N	-	-	-
196	36	M	10 Mn	2	3	2	1	2	2	A	A	12	N	-	-	-
197	26	M	1 Y	1	2	1	1	0	1	A	A	6	N	-	-	-
198	37	F	2 Y	2	2	2	2	2	2	A	A	12	N	-	-	-
199	44	M	3 Y	2	3	3	2	1	1	P	A	12	N	-	-	-
200	56	F	1 Y	1	2	1	1	0	1	A	P	6	N	-	-	-

KEY TO MASTER CHART

A	–	Absent
F	–	Female
M	–	Male
Mn	–	Month
P	–	Present
S.NO	–	Serial Number
TSS	–	Total Severity Score
Y	–	Year

Abbreviations

ABBREVIATIONS

AHT	–	Antihistamines
APST	–	Autologous Plasma Skin Test
APC	–	Antigen Presenting Cell
ASST	–	Autologous Serum Skin Test
AST	–	Autologous Serum Therapy
CIU	–	Chronic Idiopathic Urticaria
CNS	–	Central Nervous System
CVS	–	Cardio Vascular System
ELISA	–	Enzyme Linked Immuno Sorbent Assay
ESR	–	Erythrocyte Sedimentation Rate
GM CSF	–	Granulocyte Monocyte Colony Stimulating Factor
HLA	–	Human Leucocyte Antigen
HRF	–	Histamine Releasing Factor
ICAM	–	Intercellular Adhesion Molecule
IgE	–	Immunoglobulin E
IgG	–	Immunoglobulin G
INF	–	Interferon

IVIG	–	Intravenous Immunoglobulin
LT	–	Leucotriene
mRNA	–	Messenger RNA
NSAID	–	Non Steroidal Anti Inflammatory Drugs
PT INR	–	Pro Thrombin International Normalized Range
PUVA	–	Psoralein Ultra Violet A
RPM	–	Rotations Per Minute
TSS	–	Total Severity Score
UAS	–	Urticarial Severity Score
USG	–	Ultra Sonogram
VCAM	–	Vascular Cell Adhesion Molecule

INSTITUTIONAL ETHICS COMMITTEE
MADRAS MEDICAL COLLEGE, CHENNAI -3

Telephone No: 04425305301
Fax : 044 25363970

CERTIFICATE OF APPROVAL

To
Dr. S.D. Sindhuja
PG in MDDVL
Madras Medical College, Chennai -3.

Dear Dr. S.D. Sindhuja

The Institutional Ethics Committee of Madras Medical College, reviewed and discussed your application for approval of the proposal entitled " A study on chronic autoimmune urticaria and efficacy of autologous serum therapy" No. 03112010.

The following members of Ethics Committee were present in the meeting held on 24.11.2010 conducted at Madras Medical College, Chennai -3.

1. Prof. S.K. Rajan, MD	-- Chairperson
2. Prof. J. Mohanasundaram, MD,Ph.D,DNB Dean, Madras Medical College, Chennai -3	-- Deputy Chairman
3. Prof. A. Sundaram, MD Vice Principal , MMC, Chennai -3	-- Member Secretary
4. Prof R. Sathianathan, MD Director, Institute of Psychiatry, MMC, Ch-3	-- Member
5. Prof. R. Nandhini, MD Director, Institute of Pharmacology, Ch-3	-- Member
6. Prof. Pregna B. Dolia , MD Director, Institute of Biochemistry, MMC, Ch-3	-- Member
7. Prof. C. Rajendiran , MD Director, Institute of Internal Medicine, MMC, Ch-3	-- Member
8. Thiru. S. Govindasamy BA.BL	-- Lawyer
9. Tmt. Arnold Soulina	-- Social Scientist

We approve the Proposal to be conducted in its presented form.

Sd /. Chairman & Other Members

The Institutional Ethics Committee expects to be informed about the progress of the study, any SAE occurring in the course of the study, any changes in the protocol and patient information / informed consent and asks to be provided a copy of the final report

Member Secretary, Ethics Committee